ABSORPTION OF OXYGEN BY GREEN LEAVES IN RELATION TO THE INTENSITY AND SPECTRAL COMPOSITION OF THE LIGHT

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The effect of the spectral composition of light on photosynthesis was studied recently from several aspects: in connection with the spectrum of action of photosynthesis [1], by the quantum output of photosynthesis [2,3], and finally, in connection with the effect on the path of the assimilation of carbon dioxide [4,5]. In studying the latter question, it appeared that the character of the observed changes in the products of photosynthesis shows the possible activation of the oxidizing processes by short-wave radiation (400-580 mμ) in comparison with long-wave radiation (600-700 mμ). The position of the compensation points of photosynthesis is also indicated by this [6]. It is suggested that the noted changes are connected with the strengthening of absorption of oxygen by the leaf under the effect of short-wave radiation. However, experimental indications of such an effect for green leaves are practically nonexistent.

The absorption of oxygen in light was studied mainly in connection with an explanation of the action of light on respiration or photooxidation [1]. The investigation of the indicated reaction involves great methodological difficulties, because two opposing processes, the giving off of oxygen (photosynthesis) and its absorption, take place simultaneously in the green leaf in light. This difficulty is overcome either by inhibiting photosynthesis with poisons or by excluding CO_2 from the gas phase [7]. The use of the stable isotope O^{18} also opens up broad possibilities for the investigation of the question without the exclusion of photosynthesis [8].

The absorption of oxygen by green leaves in light was established with the help of the indicated methods. It appeared that both the suppression and stimulation of the process by light take place for various objects [7]. Moreover, Brown and his colleagues [9,10] recently showed that for the same object, the absorption of oxygen is changed in broad limits in relation to the intensity of the lighting: The increase in the intensity of radiation leads to a strengthening of the process.

The question of the effect of the spectral composition of the light has been much less investigated. In this connection, one can cite only the work of Foeckler [11] and Bode [12]. In the first work photosynthesis is suppressed by phenylurethan. With this a decrease was found in the absorption of oxygen on blue light in comparison to darkness; the absorption of oxygen was not observed at all for red or green light. In Bode's experiments, which were carried out in an atmosphere lacking CO_2, blue and red light activated the absorption of oxygen. Thus the intensity of the process was greater in blue light than in red light. Judging by Bode's data, blue light rather than red activates the absorption of oxygen. However, Bode and Foeckler obtained differing data on the action of red light. The light curves of the process were not taken by them. Therefore the question of the effect of the quality of the light on the absorption of oxygen by green leaves cannot be considered finally resolved.

In the experiments described below, we determined the relationship of the absorption of oxygen by green leaves to the spectral composition and intensity of light. Besides this, there was a test to explain several aspects of the mechanism of the process.

OBJECT AND METHOD OF INVESTIGATION

The experiments were carried out with leaves of tobacco, corn and beans at ages of 60, 20, and 15 days. The tobacco and corn were grown in the summer and the bean in the autumn under a DRL lamp (500 w). Leaves used in the experiment were from plants that had not undergone preliminary darkening.

The measurement of absorption in the light and dark was made with the help of the Warburg method. Slices from the leaf with dimensions of 5 cm^2 were washed in a sieve, in a cylindrical container, with a 5% KOH solution. The walls and mouth of the container were covered with alkali-soaked filter paper for more complete absorption of the carbon dioxide given off by the leaves in the process of respiration. A similar method of establishing experiments is close to that described by Frank and French [13]. The substitution of 5% KOH for 10% KOH in our experiments was justified by the fact that the more diluted alkali, as the preliminary experiments showed, significantly decreased the possibility of wilting of the leaf. The absorption of CO_2 was not decreased with this. This is shown

by the stable quantity of oxygen absorbed: of the same order as with the 10% KOH. The test containers were lighted from above with lamps placed in running water for removal of the infrared radiation. Lamps covered with a KS-II light filter served as a source of long-wave light. In the case of short-wave light, a high pressure PRK-7 mercury-quartz lamp [14] with an SZS-14 light filter was necessary.

The spectral characteristic of the light filters is presented below (Fig. 1). Use of a grid makes it possible to work with different intensities of light (see Table 1).

The lowest intensities of light in both cases gave photosynthesis below the compensation point, and the highest were below the light saturation of photosynthesis.

Experiments on the determination of the absorption of oxygen were carried out with alternating periods of dark, light and dark for a single leaf and with parallel determination of the absorption of oxygen in the dark and in the light for different sections of the leaves. It appeared that the "dark" variants on alternating dark-light-dark were somewhat differentiated from each other, that is, the after-effect of light on respiration was suggested. However, the increase of the dark respiration after lighting was unimportant (on the order of 10-15%) in comparison to the change in the activity of the absorption of oxygen under the effect of light. Therefore, in all experiments the activity of the absorption of oxygen in light was related to its absorption in the first period of dark, that is, after lighting. It is necessary to show that a leveling of the temperature conditions in the containers took place with a shift from dark to light and vice versa in a period of 30-40 min. The effect was connected with the absorption of radiation by the leaf with switching on the light and, as a result of this, the disturbance of the temperature equilibrium between the gas phase and the leaf. Therefore, we present below data obtained in conditions of temperature equilibrium without calculations for the first 30-40 min after the change in light conditions.

In the first series of experiments the relationship of the absorption of oxygen to the intensity and spectral composition of light was explained. The experimental data are shown in Fig. 2.

It follows from the data in Fig. 2 that the absorption of oxygen took place at any of the light intensities examined in the experiment. The connection of the process with the intensity of the light was clearly seen: The absorption of oxygen was greater, the higher the intensity of the light. Thus, at $3 \cdot 10^3$ ergs/cm^2 · sec (an intensity giving an amount of photosynthesis below the compensation point), the absorption of oxygen was lower than in the dark. It was, on the basis of numerous experiments, on the average not more than 40-60% of the dark. The effect of the spectral composition of light in this case already appeared as a slight increase in the absorption of oxygen under the effect of short-wave radiation relative to the long-wave radiation.

With an increase in the intensity of light, both the degree of absorption and the differences in the effect of the spectral composition of the light increased. For blue light, the activation of the absorption of oxygen by light (that is, the excess of the process over the dark) began on lighting of the order of $15 \cdot 10^3$ ergs/cm^2 · sec and about

TABLE 1. Intensity of Light in the Experiment (in ergs/cm^2 · sec)

Number of grids	Red light	Blue light
Without grid	57 700	43 300
1 grid	36 700	24 100
2 grids	19 950	13 600
3 grids	9 000	5 600
4 grids	4 000	2 800

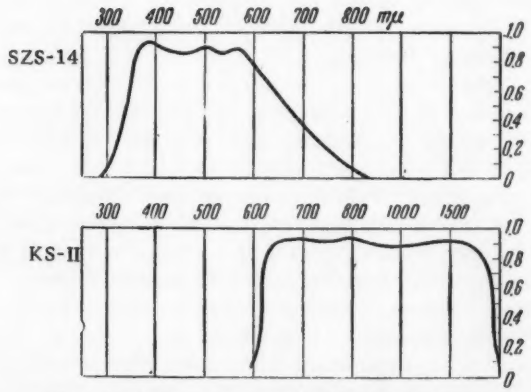

Fig. 1. Spectral curves of the transmission coefficients of optical filters.

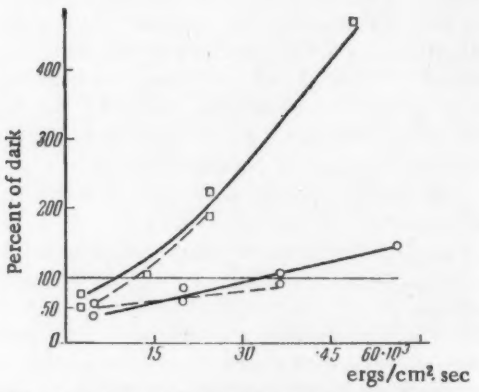

Fig. 2. Absorption of O_2 by leaves in light (as % of dark). Solid lines are for tobacco; broken lines for bean; squares on the curves are blue light and circles are red light.

TABLE 2. Absorption of Oxygen by Killed and Living Leaves in Relation to the Spectral Composition and Intensity of Light (μl O_2 per 5 cm^2/hr)

Blue light				Red light			
light intensity, ergs/cm^2 · sec	control (living leaf)	test (killed leaf)	percent of control	light intensity, ergs per cm · sec	control (living leaf)	test (killed leaf)	percent of control
2 800	15.8	None	0	4 000	5.2	None	0
13 600	33.4	5.0	14.9	20 000	9.2	"	0
24 000	32.7	6.9	21.0	36 000	8.4	"	0
24 000	27.8	2.7	10.0	—	—	—	—
47 000	51.7	11.4	22.0	57 700	19,6	3,7	18,8

$37 \cdot 10^3$ for red light. With the highest light intensities, on the order of $50 \cdot 10^3$ ergs/cm^2 · sec, the absorption of oxygen on blue light was about 300-400% and, on red light, 150-170% of that in the dark control.

Thus, it was shown that the activity of the absorption of oxygen by green leaves of plants in light does not remain constant. It is changed in relation both to the intensity and to the spectral composition of the light. In weak light, the process of absorption of oxygen is weaker than in the dark and is stronger in strong light. The activation appears more clearly in short-wave light. For leaves of the study plants, tobacco, bean, and corn, similar results were obtained, in general, showing the close connection of the process of oxygen absorption by the leaves to the light regime. A decrease in the absorption of oxygen in the absence of CO_2 in weak light in comparison to the dark usually explains the repeated use of the carbon dioxide from respiration in photosynthesis. The oxygen given off here, with a manometric determination of the gas exchange, actually must distort the results, decreasing the size of the negative effect. While not excluding this possibility, we would like to turn our attention to the fact that data similar to ours were also obtained by Kok [15] for very thick suspensions of Chlorella, and by Brown and others [16] for the algae Anabaena. Kok, like us, worked with an atmosphere free of CO_2. The experiments of Brown and others were carried out in conditions where the use of oxygen, O^{16} and O^{18}, made it possible to consider simultaneously both the absorption and giving off of oxygen. Results of work concerned with the strengthening of the reaction of the absorption of oxygen with an increase in the intensity of the light obtained by Brown and his colleagues [9,10] for algae in work with red light were similar to ours.

An explanation of the nature of the reaction is of interest. It is known that the stimulating action of strong light on the absorption of oxygen is connected with the processes of photooxidation [13,17]. Photooxidation is observed both for living and for dead boiled leaves, that is, this reaction has a nonenzymatic character. There are no data on the nature of the reaction for average and low light intensities. In order to explain the nature of the reaction in our light conditions, a second series of experiments was established. Primarily (Table 2), the size of the reaction for living leaves and leaves killed by boiling in water for one minute were compared. (With this treatment the color of the leaf did not change and no emergence of pigments into the solution was observed.)

As the data in Table 2 show, the absorption of oxygen for killed leaves at low intensities was not observed at all and some absorption was observed at the very highest intensities. Blue light aided the absorption of O_2 by the killed leaves more than red light.

However, the reaction for killed leaves never exceeded $^1/_5$ the oxygen absorption of the living leaves. Therefore, one can assume that the absorption of oxygen in our experiments, even with the highest light intensities, in general has a different nature than the effect of photooxidation.

Thus, the data in Table 2 show the enzymatic character of the process. Furthermore, the possibility of the action of the cytochrome system of the respiratory cycle in the light absorption of oxygen was explained with the help of sodium azide. The poison was introduced into the leaf by the method of vacuum filtration following freeing of the intercellular spaces of water. The latter was necessary in connection with the fact that the absorption of oxygen in light appeared to be more responsive in relation to aeration conditions than the absorption of oxygen in the dark. The greater the degree of moisture in the leaf, the less the reaction as compared to the dark (Table 3). While for normal, turgescent leaves not treated with water, activation of the absorption of oxygen by blue light on the order of 70% was observed, leaves floating in water for 22 hours did not show an excess of the process as compared to the dark. For infiltrated leaves (without elimination of water) absorption was even lower than in the dark (60% of the dark). Elimination of intercellular spaces to a significant degree restored the size of the reaction. At the same time, in spite of the decrease in the reaction, the usual character of its reaction to the light conditions (intensity and spectral composition) was maintained (Table 3).

The action of sodium azide on the absorption of oxygen in the dark and in short-wave light ($36 \cdot 10^3$ ergs/cm_2 · sec) is shown in Fig. 3. Sodium azide in concentrations of $7 \cdot 10^{-4}$ moles significantly (up to 40-50%)

TABLE 3. Relationship of Light Absorption of Oxygen to the Degree of Soaking of the Leaf

Leaf preparation	Activity of absorption of oxygen in light as % of dark	
	Blue light, $24 \cdot 10^3$ ergs/cm^2 · sec	Red light, $36 \cdot 10^3$ ergs/cm^2 · sec
No preparation	170	135
In water 22 hours	103	58
Immediately after infiltration	66	–
40 minutes after infiltration; intercellular spaces $^2/_3$ eliminated	133	–

TABLE 4. The Effect of Sodium Azide on the Absorption of Oxygen by Tobacco Leaves in Relation to the Spectral Composition and Intensity of Light

(Data in µl O_2 per 5 cm^2/hr)

Conditions of illumination	Strong light (one grid)		Weak light (four grids)	
	control	NaN_3 $7 \cdot 10^{-4}$ M	control	NaN_3 $7 \cdot 10^{-4}$ M
Dark	17.3	7.4	18.3	9.7
Blue light	22.3	20.2	3.2	3.7
Dark	25.4	10.1	–	–
Dark	21.4	11.7	10.6	6.9
Red light	14.5	14.6	3.4	2.6
Dark	20.0	13.8	–	–

suppressed the absorption of oxygen in the dark. Consequently, a significant portion of the dark respiration for tobacco leaves takes place in proportion to the metal-containing oxidase.

Differences between the control and experimental leaves were not observed on short-wavelength light – the poison did not decrease the absorption of oxygen. The absence of an effect of the poison was connected with its disintegration. Turning off the light reestablished the previous picture in dark again, even to a greater degree than earlier; a suppression of the reaction was observed.

The effect of sodium azide was also investigated in relation to the intensity and spectral composition of the light (Table 4). At high intensities of both the short-wave and the long-wave radiation, sodium azide practically did not affect the absorption of oxygen. The same can be also said of the effect of weak light. Thus, the data in Table 4 make possible the usual conclusion that the absorption of oxygen in light is insensitive to the sodium azide in those concentrations that suppress dark respiration. From this it follows that the nature of the enzymes taking part in the absorption of oxygen, and the general mechanism of the reaction for the green leaf, are different in light and dark. Of course, part of the absorption of oxygen in light obviously is accomplished according to the dark type with the action of oxidases of the respiratory cycle localized in the nongreen parts of the leaf – plasma and mitochondria. However, at high illuminations this portion of the absorption of oxygen is insignificant.

According to the data in the literature [1], it is known that sodium azide inhibits not only respiration but also the photosynthetic generation of oxygen. We attempted to calculate the absorption and generation of oxygen in the light by means of selecting the concentration of sodium azide. In the experiments carried out below, we compared the susceptibility to the azide of three processes: the generation of oxygen on photosynthesis, the absorption of oxygen in light and in dark. Sodium azide is infiltrated into the leaf in different concentrations. Then slices were placed in an atmosphere containing CO_2 (Warburg buffer No. 9 was added at the mouth of the container along with the KOH).

Results that were obtained are presented in Table 5.

The poison in a $7 \cdot 10^{-4}$ mole concentration did not affect the light absorption of oxygen, and completely suppressed photosynthesis and respiration at 60% of darkness (experiments 1 and 2). At a poison concentration of $5 \cdot 10^{-4}$ mole the dark respiration was not suppressed; however, photosynthesis in this case was also completely suppressed (experiment 3). This can also be stated for experiment 4 (poison concentration of $2.5 \cdot 10^{-4}$M). The results that are presented give an idea of the different susceptibilities to the action of sodium azide of oxidases of the respiratory cycle, the process of oxygen generation with photosynthesis and the process of light absorption of oxygen. The most susceptible appeared to be the reaction of oxygen generation. Using different susceptibility of the indicated reactions to sodium azide, one can study the reaction of oxygen absorption in the presence of CO_2. With this the possibility of distortion of the indicators in manometers is eliminated in proportion to the summation of the two simultaneously proceeding processes, the absorption and generation of oxygen.

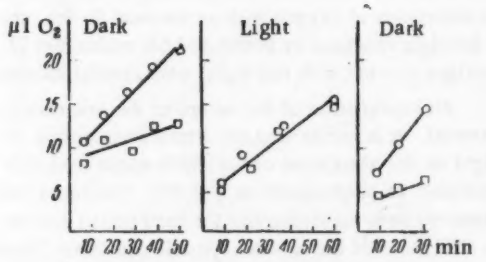

Fig. 3. The effect of sodium azide on the absorption of O_2 in light and in dark. In µl O_2 per 5 cm^2/hr. Circles on the curves are controls; squares are experimental results.

TABLE 5. The Effect of Sodium Azide on the Absorption and Generation of Oxygen by Tobacco Leaves in Light (Data in μl O_2 per 5 cm^2 per hr)

Expt. no. and molarity of NaN_3	Illumination conditions (light intensity in erg/$cm^2 \cdot$ sec)	Absorption (−) and generation (+), μl O_2 per 5 cm^2/hr	
		control	test
1 $7 \cdot 10^{-4}$	Blue light $47 \cdot 10^4$	+25.5	−13.9
2 $7 \cdot 10^{-4}$	Dark	−16.3	−7.3
	Blue light $24 \cdot 10^4$	+14.8	−13.8
3 $5 \cdot 10^{-4}$	Dark	−10.8	−10.5
4 $2.5 \cdot 10^{-4}$	Blue light $47 \cdot 10^4$	+33.4	−10.1
	Red light $36.7 \cdot 10^4$	+13.1	−10.0

DISCUSSION OF THE RESULTS

The data that were obtained show that for green leaves in light the absorption of oxygen different from that in the dark is observed. The intensity of this absorption is determined by the light intensity and depends on its spectral composition. With low exposure, the light absorption of oxygen is even less than in the dark; the increase in the intensity of light, both of long-wave and of short-wave light, leads to the activation of the process. Our results for higher plants in this respect completely correspond to the results [9,10] obtained for photosynthesizing algae with the use of isotopes of oxygen and carbon dioxide. In these works and in our experiments, the objects of investigation were different – lower and higher plants. However, because of the presence of chlorophyll they possessed one common characteristic: the capacity for photosynthesis. The same common characteristic of green plants, apparently, is the relationship of the absorption of oxygen to the illumination conditions. At the same time, this process to some degree does not depend on the assimilation of CO_2 by the leaf, insofar as it is observed both with photosynthesis and in its absence, regardless of whether or not the process of photosynthesis is suppressed by poison or by the exclusion of CO_2.

The spectral composition of the light has a significant effect on the size of the reaction: With an increase in the quantity of radiation the absorption of oxygen increases in both regions of the spectrum; however, in the case of short-wave radiation, the process proceeds significantly more rapidly. The latter supports the idea described earlier, that the strengthening of the oxidizing processes under the effect of short-wave radiation is connected with the absorption of oxygen [6].

The results obtained for green leaves differed from the results obtained by us earlier on the action of light on the absorption of oxygen by chlorophyll-noncontaining leaves. For the latter, any intensity of blue light activated the process relative to the dark, but red light did not show any effect [18]. The enzyme taking part in the absorption of oxygen in light by green leaves is not, as for nongreen leaves, the cytochrome oxidase of the respiration cycle, as we had supposed earlier [6]. This enzyme is differentiated by its high resistance to sodium azide.

Insofar as the differences are determined by the presence of chlorophyll, it is obvious that the systems with the help of which the absorption of oxygen by green leaves in light takes place are localized in the chloroplast. It is possible that these components of the cytochrome system differ in properties from the cytochromes of the respiration cycle. In connection with the investigations in which the localization of a whole series of cytochromes in the chloroplast was established, there is now a sufficient basis for this assumption [19,20,21].

Thus, it is possible that photooxidase recently found in chloroplasts of spinach and other green plants is responsible for the absorption of oxygen in light [22,23,24]. As in our experiments, this photooxidase, oxidized by cytochrome C in light, was not susceptible to those concentrations of sodium azide that suppressed the action of the oxidases in the dark. This enzyme was not found for etiolated leaves. According to modern ideas, the cytochrome systems, being components of the photoactivated complex of the chloroplasts, takes part in the transfer of energy absorbed by the chlorophyll. The close connection of the enzyme to the chlorophyll and the possibility of migration of energy into the complex make it possible to explain the effect of the intensity of light on the process of oxygen absorption and the differences in the action of the short-wave and long-wave radiation: The activity of the oxidase in this case is in direct relation to the quantity of light absorbed by the chlorophyll. The action both of the red and of the blue light can conform to this rule. However, in the case of short-wave radiation, additional activation of the process can take place in proportion to the immediate absorption of light by the enzyme itself. Indirect support of this can be found in the work of Krall [26]. He showed that the photosynthetic phosphorylization activated those parts of the short-wave radiation that are absorved by the cytochromes. Sisakyan, Krasnovskii and others [27] observed the decomposition for isolated chloroplasts of the complex of cytochrome oxidase with CO not only by white light, containing short-

wave rays, but by red light, although to a lesser degree. This is connected also with the possibility of a transfer of energy of light absorbed by the chlorophyll to other systems.

The question of the paths of the rise of the absorbed oxygen of the photosynthesizing cell at the present time is not clear. It is obvious only that this process is to a significant degree unrelated to the intensity of the reaction of the respiration cycle. In fact, the probable localization of it in the chloroplasts makes doubtful its part in the respiratory conversions. The overwhelming majority of work showed that light does not have an activating effect on the generation of CO_2 or that it suppresses this process [28].

A number of facts established by the scientific associations directed by Nichiporovich [29,30] and also the data of Moses and others [31] and Moiz [32] show the difference in the metabolism of green plants in the light and in the dark.

Although some portion of the absorbed oxygen possibly is also connected with the reactions of the respiration cycle, the normal figure for the absorption of oxygen in clear light significantly exceeds (especially in the case of short-wave light) the demands for oxygen for respiration conversions. The reactions in the lighted leaf that can be connected with the absorption of oxygen must be shown in further investigations. At the present time, analysis of the existing literature makes it possible to state only the proposition that the light absorption of oxygen can be specified by reactions with recovered water obtained as a result of photooxidation. Now, sufficient data are accumulated on the possibility of the photochemical restoration by photosynthesizing plants of such substances as NO_3, NO_2, and SO_3, besides CO_2. [32,33,34]. With this, the competitive character of the process of assimilation of nitrates and CO_2 in photosynthesis is revealed [31]. Brown [9] suggests that there can be an interaction between the reducer and the atmospheric oxygen. According to his data, the less the concentration of CO_2, that is, the less reducer needed in the restoration of CO_2, the more actively the absorption of oxygen takes place. For green algae and Chlorella, the possibility of oxidation of photochemically formed reducer not only by oxygen, but also by hydrogen peroxide, was established [35]. In studying Hill's reaction in an atmosphere of normal air, the absorption of oxygen by fragments of the chloroplasts was indicated [36]. The size of this absorption depended on the activity of the Hill reaction, that is, it was closely connected with the photooxidation of water. The possibility of the absorption of atmospheric oxygen, depending on the photooxidation of water, is supported by experiments by Mehler [37] with the O^{18} isotope of oxygen.

Of course, other uses of absorbed oxygen are not excluded (for example, on the change in the relationship of the products of photosynthesis, strengthening of the reaction of phosphorylization, etc). It is possible that for aerobic autotrophic plants, this process is necessary for maintaining in some condition a certain oxidation-reduction regime of photosynthesizing cells [38,39].

SUMMARY

1. The intensity of oxygen absorption by green leaves in light depends on the intensity and spectral composition of the light.

An increase in the intensity both of long-wave and of short-wave radiation activates the process. However, in the case of short-wave light the process proceeds more intensively.

2. The oxidase taking part in the absorption of oxygen by leaves in the light differs in its susceptibility to sodium azide from the oxidase of the respiration cycle.

3. The data that were obtained show the differences in the mechanism of oxygen absorption by green leaves in the light and dark.

LITERATURE CITED

1. E. Rabinowitch, Photosynthesis [Russian translation] (IL, 1951).
2. R. Emerson, Ann. Rev. of Plant Physiol. 9, (1958).
3. O. Warburg, G. Krippahl, W. Schröder, W. Buchholz and Z. Theel, Naturforsch 9b, 164 (1954).
4. N. P. Voskresenskaya, Fiziol. Rastenii 3, 49 (1956).
5. T. Cayle and R. Emerson, Nature 179, 4550, 89 (1957).
6. N. P. Voskresenkaya, Problems of Photosynthesis [in Russian] (Izd. AN SSSR, 1959).
7. R. A. Weintraub, Bot. Rev. 10, 7, 383 (1944).
8. E. Rabinowitch, Photosynthesis [Russian translation] (IL, 1959) Vol. 3.
9. A. H. Brown and D. Weis, Plant Physiol. 34, 3, 224 (1959).
10. D. Weis and A. H. Brown, Plant Physiol. 34, 3, 235 (1959).
11. H. Föckler, Jahrb. wiss. Bot. 87, 45 (1938).
12. O. Bode, Jahrb. wiss. Bot. 89, 2, 208 (1940).
13. J. Frank and C. S. French, J. Gen Physiol. 25, 309 (1941).
14. Handbook on Light Techniques [in Russian] (Izd. AN SSSR, 1956) Vol. 1, pp. 388-389.
15. B. Kok, Symp. Soc. Exptl. Biol. 5, 211 (1951).
16. A. H. Brown and G. C. Webster, Amer. J. Bot. 40, 753 (1953).
17. J. Myers and G. O. Burr, J. Gen. Physiol. 24, 45 (1940).
18. N. P. Voskresenskaya and E. G. Zak, Doklady Akad. Nauk SSSR 114, 2, 375 (1957).*
19. R. Hill and R. Scarisbrick, New Phytologist 50, 98 (1951).
20. L. Smith and B. Chance, Ann. Rev. of Plant Physiol. 9, 449 (1958).
21. E. F. Hartree, Cytochrome in Higher Plants. Advances in Enzymology 28, 1, 7 (1957).

* See English translation.

22. R. H. Nieman and B. Vennesland, Plant Physiol. 34, 3, 255 (1959).
23. R. H. Nieman, H. Nakamura, and B. Vennesland, Plant Physiol. 34, 3, 262 (1959).
24. N. I. Bishop, H. Nakamura, J. Blatt, and B. Vennesland, Plant Physiol. 34, 5, 551 (1959).
25. B. Chance and M. Nishimura, Proc. Nat. Acad. Sci. USA 46, 1, 19 (1960).
26. A. R. Krall, Research in Photosynthesis (New York, 1957).
27. N. P. Sisakyan, A. A. Krasnovskii, E. S. Mikhailova, and T. P. Brin, Biokhimiya 24, 3 (1959).
28. O. V. Zalenskii, Bot. Zhur. 42, 1672 (1957).
29. A. A. Nichiporovich, T. E. Andreyeva, N. P. Voskresenskaya, L. A. Nezgovorova, and U. L. Novitsky, International Conference of Radioisotopes in Scientific Research, UNESCO (NS) RJCI23 (Paris, 1957).
30. A. A. Nichiporovich, Problems of Photosynthesis [in Russian] (Izd. AN SSSR, 1959).
31. V. Moses, O. Holinhansen, J. A. Bassham, and M. J. Calvin, Molecular Biology 1, 21 (1959).
32. A. Moiz, Fiziol. Rastenii 6, 274 (1959).*
33. E. Kessler, Planta 49, No. 5, 505, (1957).
34. N. G. Doman, Biokhimiya 22, 716 (1957).
35. T. Hirokawa, S. Miyachi, and H. Tamiya, Biochem. 45, 12, 8 (1958).
36. T. F. Andreeva and L. E. Zubkovich, Doklady Akad. Nauk SSSR 60, 4, 681 (1948).
37. A. H. Mehler and A. H. Brown, Arch. Biochem. and Biophys. 38, 365 (1952).
38. A. A. Nichiporovich, Proceed. Internat. Conf. on Peaceful Uses of Atomic Energy 12, (1956).
39. A. A. Nichiporovich and N. Voskresenskaya, Proceed. Internat. Botanical Congress, Montreal, II, Abstr. (1959).

*See English translation.

RATE OF PHOTOSYNTHESIS FOR CORN OF DIFFERENT VARIETIES

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The introduction of corn into new regions quite naturally must be supported by the study of the various aspects of its behavior under given ecological conditions. The selection of average-ripening and average-late-ripening varieties with a high yield of green mass and ripe ears is of great importance here.

In the absence of special varieties created by means of selection, the problem of corn for new zones can be solved by the selection and detailed study of the available varieties. The latter is especially necessary in cultivating corn from seeds. A number of methods, such as simultaneous (early) sowing, knowledge of variety peculiarities, and selection acclimatization, etc.,to increase early ripening, can be employed by agronomists when local seeds are available.

Corn occupies an important place in the fodder balance in the Zaural region, Western Siberia, and other regions of virgin soil and waste lands with their primarily chernozem soils. The indicated zone has a very continental and arid climate and an adequate amount of warmth. The goal of the study of corn should be not only to determine which varieties are early ripening and give high yields, but also to establish the drought resistance of these varieties.

One of the indicators of these characteristics in corn is the rate of photosynthesis, which is an important physiological process and a basic factor of the yield. Therefore, the study of photosynthesis for various varieties in the new habitat conditions is of interest in connection with the insufficiency of data in the literature on the photosynthesis of corn.

Our investigations on this question were carried out at the Troitsk educational-experimental farm of Permsk University (southern part of the forest steppe of Chelyabinsk region) in the 1957-1958 period. The hydrothermic conditions of the indicated years in the indicated zone differed significantly. The summer of 1957 had an average monthly temperature of 15.8° and a total precipitation of 245 mm. In spite of drought at the beginning of the summer, with an abundance of moisture and heat in July and August and a late frost (September 24), the conditions for the growth and development of corn were completely favorable.

The summer of 1958, with an average temperature of 13.5° and a total precipitation of 199 mm, was distinguished by sharp and continuing drought, because September precipitation (105mm), with low temperatures, was practically ineffective. Because of this, and also because of an early frost (September 10), this year was extremely unfavorable for corn. The indicated meteorological conditions had a great effect on the rate of photosynthesis and all behavior of the corn varieties.

1. Experimental Conditions and Calculation of Photosynthesis for Different Varieties of Corn, 1957

The variety study of corn in 1957 was carried out on a weakly leached clay chernozem with complete fertilization applied under a cultivator plow. Sowing was done on May 10 with a normal sowing of 16-25 kg of seeds per hectare in relation to the size of seed. Ten varieties were tested, three of which were obtained from Troitsk elevators, the others having been grown locally in previous years. The survival of 30-32 thousand plants per hectare was guaranteed.

Varieties with primarily average- and late-ripening forms were taken to study photosynthesis; brief biometric and phenologic data are presented in Table 1.

Calculation of photosynthesis was made for corn of six varieties once every ten days by the method of determining carbon according to I. V. Tyurin in the Borodulina and Kolobaeva modifications [1] in discs of specific size taken in the morning and evening from a single leaf. The fourth (or fifth) leaf from the top, the one most completely formed and most active, was always taken for measurement. The amount of daytime accumulation of carbon by the leaves in mg/dm^2 for 12 hours for the given day and variety was established according to the differences in the concentration of carbon in the morning and evening samples.

The indicator of the intensity of photosynthesis is usually expressed in the literature [2-7] in the form of mg CO_2/dm^2 per hour assimilated by the leaves. The object of the investigations and the conditions of field work did not allow us to use the latter method, which is poorly adapted for calculation of the rate of the process.

The daily accumulation of carbon (or intensity), as is well known, is closely connected to the rate of treat-

TABLE 1. Biometric and Phenologic Data for Corn of Different Varieties in the 1957 Season on July 31

Variety	Height, cm	No. of nodes per plant	Leaf length, cm	Leaf width, cm	Percent of flowering tassels on July 20	Percent of pollen on July 20	No. or % of ears per 100 plants on August 21
Hybrid VIR-42	246	14	76.5	8.6	24	14	150
Sterling	256	12.8	79.5	9.2	40	18	138
Yellow, Stalinsk region	231	12.1	64.7	8.5	15	13	160
White Kharkov	238	12.6	85.8	8.4	71	76	156
Yellow Rumania	238	12.5	72.2	10.7	54	41	165
North Dakota	198	9.9	75.9	7.9	36	127	171
Karabalyk, early ripening	150	10.1	43.1	5.9	90	197	220
Number of measurements	25	25	100	100	100	100	100

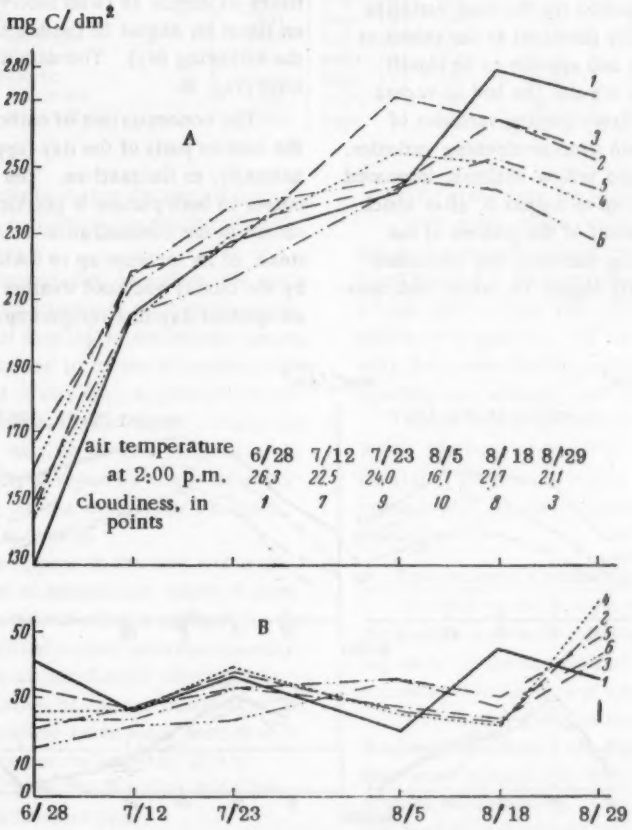

Fig. 1. Rate of photosynthesis for corn of different varieties by date, 1957 season. A) Concentration of carbon (C) per 1 dm² of leaf surface (morning calculation); B) daily accumulation of carbon. 1) Hybrid VIR-42; 2) Yellow Stalinsk; 3) Sterling; 4) Nor- North Dakota; 5) yellow Rumania; 6) Karabalysk flint.

ment and flow of assimilates and the intensity of respiration, and strongly depends on the weather conditions of the day. In this connection we considered it necessary to trace additionally the rate of the normal concentration (accumulation) of carbon in the leaves over the vegetation period. This quantity appeared to be more stable; in a number of cases it can play in like manner an important independent role. Both indicators, especially the first, are identical with the "pure productivity of photosynthesis" [4].

The usual level of the indicated figures for the different varieties and its change by dates of vegetation are shown in Fig. 1.

Curves of the normal concentration of carbon by dates as an indicator of the process over an extended period, after an intensive increase began to decline at the end of August for all varieties, a fact explained by the growth properties of the plants and the decrease in soil moisture (from 18 down to 12% average per cut).

The concentration of carbon for the flint varieties (curves 5 and 6) is practically identical to the others at the beginning of vegetation and appears to be significantly lower (by 25-30 mg) towards the end of vegetation than for the tooth-like late-ripening varieties of corn. The early-ripening and average-ripening varieties, Karabalyk, North Dakota, and yellow Stalinsk, increased the concentration of carbon up to August 5, after which a decrease was noted as a result of the process of ear formation. For the remaining varieties, the concentration of carbon increased until August 18, which indicates their late ripening.

The curves of the daytime accumulation of carbon, the "pure productivity of photosynthesis," have a different character over the period of a summer. The figure showing the daytime assimilation by the calculation of all expenditures of carbon by the plants varies on different days.

The behavior of different varieties, based on the daytime accumulation of carbon, is less constant and regular. The greatest average daily productivity of photosynthesis (over a season) is observed for the varieties hybrid VIR-42, yellow Stalinsk, Rumania, and North Dakota, which give the highest yield of green mass and ears (see Table 3). The exception in this case was variety Sterling, the decreased photosynthesis and high yield of which still have not been explained.

Further determination of the characteristics for the four varieties was carried out by measuring the daily rate of photosynthesis, which is of interest for these conditions. The indicated calculation was made twice; six times on August 18 (with intervals of four hours) and seven times on August 29 (with a check in the morning of the following day). The data are presented in graphic form (Fig. 2).

The concentration of carbon in the leaves during the various parts of the day (upper curves) is subject, naturally, to fluctuations. The usual character of the curves in both periods is practically the same. A decrease in the concentration of carbon in the leaves instead of its increase up to 9 AM on August 18 is caused by the cloudy and cool weather in the morning. With an optimal daytime temperature (22°), photosynthesis

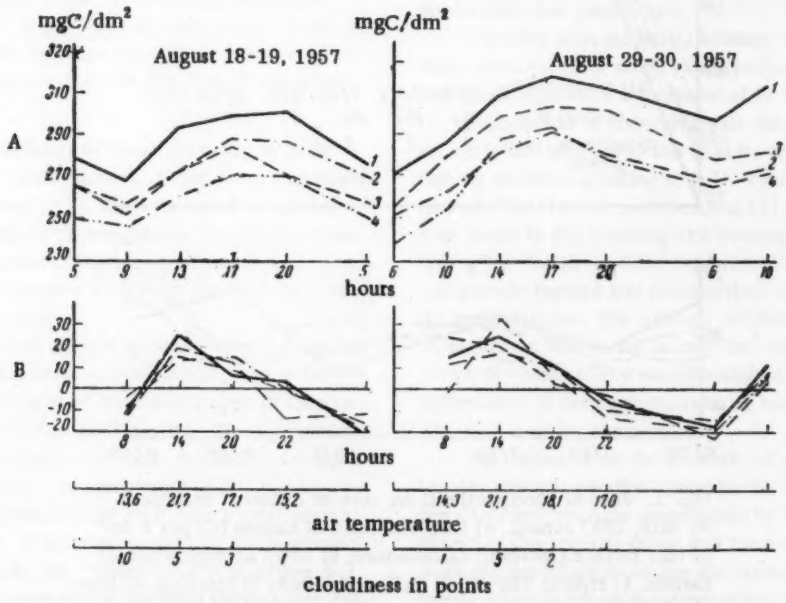

Fig. 2. Daily path of photosynthesis of corn. A) Level of carbon (C) concentration in leaves; B) accumulation of carbon over the period of a day. 1) Hybrid VIR-42; 2) Sterling; 3) yellow Stalinsk; 4) North Dakota.

TABLE 2. Rate of Concentration of Carbon in Corn of Different Varieties (August 29-30, 1957)

Variety	Time of calculation (hour)						
	6	10	14	17	20	6	10
Hybrid VIR-42	271.4	286.9	307.1	318.2	312.6	295.1	305.7
Sterling	251.7	263.8	280.6	291.4	281.9	266.9	273.7
Yellow Stalinsk	263.8	279.0	299.1	303.3	300.5	277.2	281.4
North Dakota	237.6	255.9	287.0	293.8	279.8	263.6	269.9
Air temperature	—	14.3	21.1	18.1	—	—	—
Cloudiness in points	—	1	5	2	—	—	—

TABLE 3. Yield of Corn of Different Varieties, 1957 Experiment

Variety	Year of first reproduction	Date of harvest	Wt. of silage mass in centners/ha on 8/20	Wt. of ripened ears, centners per ha	Average weight per ear, g	Weight per 1,000 grains
Hybrid VIR-42	1957	Sept. 20	364	72.5	283	290
Sterling	1957	Sept. 19	358	74.1	270	314
Yellow Stalinsk	1956	Sept. 19	318	66.2	197	280
White Kharkov	1956	Sept. 16	326	69.4	226	296
North Dakota	1956	Sept. 12	280	62.6	202	312
Yellow Rumania	1956	Sept. 16	345	70.0	199	280
Yellow Karabalyk	1950	Sept. 2	165	38.4	108	188

takes place most productively during the period from 1 PM to 5 PM, after which a decrease for practically all varieties is observed.

According to the concentration of carbon, the varieties are located in an order inverse to their early ripening, with the exception of Sterling in the second period. This same order is maintained in curves of accumulation of carbon over the period of one day, August 18 (lower series). At the end of August, that is, at the time of the intensive ripening of the ears, in general an increase in assimilation during the day is noted for the more early-ripening varieties (North Dakota and yellow Stalinsk), although this increase is not sharp.

Variety peculiarities appear in the rate and quantity of the nighttime flow of assimilates, which is seen on August 29, when the accumulation is changed by the consumption of carbon. In individual cases the consumption exceeds the daytime accumulation, obviously in proportion to the assimilation of the preceding day. In general the decrease in carbon takes place from 6 to 7 AM, with its following increase only at 9 to 10 AM (Table 2). This was also noted by Bazyrina and Chesnokov [8] for potatoes, radish and oats.

The most intensive flow of assimilates from the leaves (into the ears) was noted for North Dakota, which coincides with the earliest ripening of this variety.

During the second half of September, the corn was harvested in relation to its ripening with a calculation of the normal yield and of the ripe ears individually (green mass was calculated earlier by 10-meter squares).

The yield data are presented in Table 3.

With early sowing against a background of fertilizer with good and varied types of care of the plants, in conditions of a favorable year, we managed to obtain a high yield of ripe ears, which suggests the good possibilities of growing corn for seed in this area for not only the early-ripening varieties, but also the average-ripening and average-late varieties.

North Dakota, which ripens prior to the onset of frosts, can be considered the best of the varieties for obtaining grain and average yields of silage. After this come white Kharkov, yellow Stalinsk and yellow Rumania.

2. Experimental Conditions and Results, 1958

In 1958 we studied 12 varieties from our 1957 reproduction that were different in rate of ripening. Sowing them on the same plots against a background of secondary fertilizers was carried out on May 6 and 7.

The corn sprouts (in connection with the cold weather) appeared during the period from May 22 to 25; they were damaged by frost on May 28, but remained without transplanting. The following varieties suffered less than the others and recovered more rapidly: yellow Rumania, yellow Stalinsk, white Kharkov and the early-ripening varieties.

In spite of good care, the corn of all varieties did not reach its average growth. Drying of the soil, as a result of which its moisture in the second half of August fell to 8-9%, caused a chronic wilting of the leaves,

retardation with dropping of the stigmas and a sharp suppression of growth and development.

Investigations of photosynthesis in 1958 were carried out on three new varieties: Uspekh, Rumania and Spasovskaya. High resistance to drought was observed for variety Uspekh with late beginning development: it practically did not undergo wilting even in August; its thick leaves remained green up to the end, in contrast to the other varieties. For Rumania corn, the effect of many years of selection was the earlier ripening of this normally late-ripening variety.

We attempted to trace variety characteristics of corn in 1958 by the daily path of photosynthesis for six varieties during three periods of the season by means of six calculations of the concentration of carbon per day. The data for this calculation by the period of the day is presented in graphic form (Fig. 3).

An accumulation of carbon took place during the period on July 12 at an optimal temperature of 24°, but on August 4 between 1 PM and 5 PM at 28°, a decrease in its level was noted (not observed in 1957), which apparently is connected with the overheating and drying of the leaf cells. A reduction of photosynthesis in corn was noted by Shkol'nik and Makarova [6] and other authors in the more southern regions of the country. The concentration and accumulation of carbon over the period of a day was the highest on August 22, in connection with cloudiness and increased air moisture. First again in this respect on different days and hours of the day is North Dakota, then Uspekh, Spasovskaya and Rumania. Based on the daily accumulation of carbon proceeding in general similarly to the level of the concentration, a similar increase in the following period is observed (data are not presented).

The highest productivity of photosynthesis in the first period among the varieties was observed for Spasovskaya and Sterling, in the second for varieties Uspekh and Spasovskaya, and in the third for North Dakota, Uspekh and Rumania.

The data that were obtained again show that photosynthesis proceeds on early dates and more intensively for early-ripening varieties. Close to this is variety Uspekh (average-late ripening), apparently as a result of its drought resistance. The other late varieties, because of the drought, could not raise the level of photosynthesis to the average norm for the second half of the summer which we observed in 1957. Thus, the peculiarities of corn varieties, on the basis of the productivity of photosynthesis, were less clearly differentiated in the year 1958.

A different picture is given by the calculation of the productivity of photosynthesis in kilograms of carbon per hectare by the A. A. Nichiporovich method, with the recalculation of the amount of daily accumulation for the indicated variety on the average leaf area of a single plant and the average number of plants per hectare, in the different periods of the season. The indicated biometrics were carried out one or two days immediately after the calculation of photosynthesis, and in the third period simultaneously with the latter. The data on the quantity of carbon accumulated after a day by different varieties per hectare by periods are presented in Table 4.

The leaf area, which increases greatly during July and the first half of August, is sharply decreased towards the end of the month for all varieties except variety Uspekh. However, the total accumulation of carbon in the following period is higher for all varieties except flint Spasovskaya, which has completed development.

The data of the second period appeared to be low and irregular as a result of the high temperature and the midday depression, and also in connection with the flowering phase. The difference between the varieties, based on the accumulation of carbon in the first and third periods, is most clear and regular: Higher productivity is

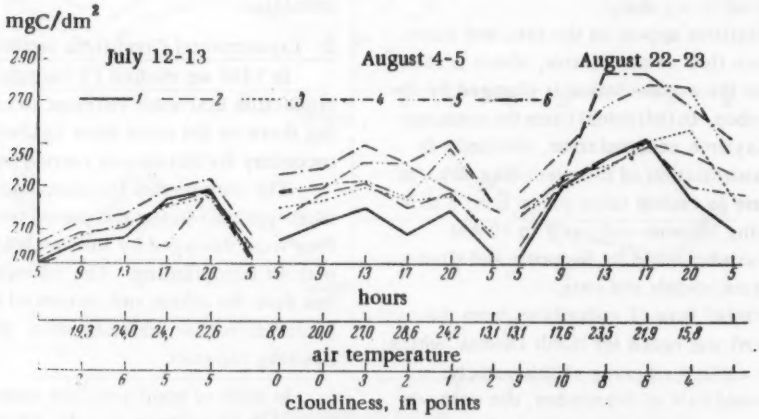

Fig. 3. Daily path of photosynthesis for different varieties of corn (dynamics of the level of carbon in the leaves). 1) Sterling; 2) hybrid VIR-42; 3) yellow Rumania; 4) North Dakota; 5) Spasovskaya flint; 6) Uspekh.

TABLE 4. Leaf Area and Accumulation of Carbon for Corn of Different Varieties in the 1958 Season

Variety	July 12		August 4		August 22	
	leaf area, thousands of m²/ha	carbon kg/ha	leaf area, thousands of m²/ha	carbon, kg/ha	leaf area, thousands of m²/ha	carbon, kg/ha
Sterling	5.28	17.93	9.62	13.47	8.47	24.22
VIR-42	6.82	18.29	8.82	11.18	7.93	34.86
Yellow Rumania	6.53	18.64	9.50	6.37	8.31	32.81
Uspekh	—	—	12.05	24.44	11.95	61.06
North Dakota	5.02	14.96	6.80	10.68	5.03	16.75
Flint Spasovskaya	4.08	13.86	4.94	1.45	3.64	5.06

Note: 1. See Fig. 3 for the meteorological conditions for the days. 2. The number of plants per hectare for all varieties on the average was 23,200.

TABLE 5. Yield of Ears for Different Varieties of Corn, 1958

Variety	Number of plants per hectare, 1000's	Yield of ears without husks	
		total, centner/ha	hard ripened, centner/ha
Sterling	24.0	11.7	4.00
VIR-42	24.0	18.8	4.69
Yellow Rumania	24.1	20.6	11.1
Uspekh	22.0	20.8	7.26
North Dakota	22.3	22.1	12.63
Flint Spasovskaya	23.02	18.20	12.45

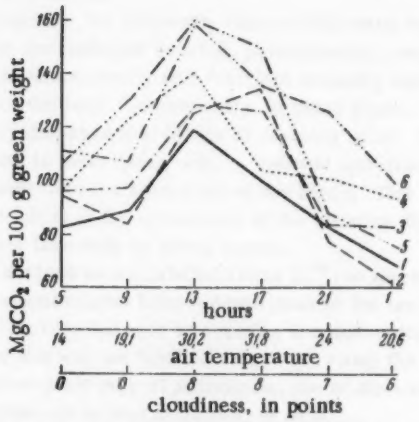

Fig. 4. Daily respiration path for leaves of different varieties of corn. Measurements on August 16, 1958. 1) Sterling; 2) hybrid VIR-42; 3) yellow Rumania; 4) Uspekh; 5) North Dakota; 6) flint Spasovskaya.

shown for the late-ripening varieties than for the early-ripening varieties. However, in the conditions of 1958, for corn of average-late varieties, this advantage was not realized in the normal harvest and especially in the ears.

The daily dynamics of the intensity of respiration of the varieties of corn studied was determined on August 16 by the Boisen-Jensen method. The high temperature (20-32°) and a clear sky led to the great energy of this process (Fig. 4).

The intensity of respiration of the early-ripening varieties appeared to be significantly higher during all hours of the day than for the remaining varieties, which confirmed the existence of variety peculiarities for the plants and the difference in the phases of their development in the indicated period.

The water deficit for the plants in the midday hours, which caused a depression in photosynthesis and other processes, clearly appeared in the concentration of water in the leaves, which during the day increased to 12-13%.

The higher resistance to drought of the early-ripening and average-ripening varieties was reflected in the high yield of ripe ears, the data for which on September 13 are presented in Table 5.

The early-ripening varieties with fewer plants per hectare gave an average harvest of 12 centners of ripe ears, which can be considered satisfactory for 1958. Rumania and Uspekh were close to it with 11 and 7 centners. The remaining average-ripening varieties (VIR-42 and Sterling) appeared to be little suited to the severe conditions of the given year and gave a low yield of ripe ears.

SUMMARY

1. Varieties of corn differing in the speed of ripening are differentiated by the total level and character of the accumulation of carbon after vegetation. Photosynthesis for early-ripening varieties (and the phases of development) proceeds more intensively on early dates, reaching a maximum (based on the total concentration of carbon) in July and then decreasing; the maximum for late-ripening forms is attained in August. The average (for the season) daily amount of accumulation of carbon

is 25-30 mg higher for late-ripening varieties than for early-ripening.

2. In very dry conditions (1958), the rate of photosynthesis by periods appeared to be disturbed and the difference between varieties was less distinct. However, the late-ripening varieties also clearly showed a higher productivity than the early-ripening varieties, based on the total accumulation of carbon after 12 hours in kg/ha (with the calculation of leaf area).

3. Numerous calculations of the daily path of photosynthesis for the different varieties indicate that it proceeds for 17 or 18 hours, and the fluctuations in the accumulation of carbon over the period of a day. The rate and size of the nighttime flow of assimilates from the leaves appeared to be higher (up to the end of August) for early-ripening varieties. The intensity of respiration of the latter in all hours of the day also was significantly higher than for the remaining varieties.

4. The indices of photosynthesis for July and August reflect (and determine) the variety peculiarities of corn; in the majority of cases they are correlated with the size of the total harvest (and with ripe ears) and can serve as comparative indices in characterizing and evaluating the productivity of different varieties of corn.

5. The late-ripening, high-stemmed variety with a high yield of ripe ears in 1957 had a sharply decreased yield in 1958. To guarantee the yield of seeds of corn in the given zone, the annual cultivation of a number of varieties with different dates of ripening and the methodical practice of systematic procedures for the accumulation and retention of moisture in the soil are necessary.

LITERATURE CITED

1. F. Z. Borodulina and L. G. Kolobaeva, Doklady Akad. Nauk SSSR 90, 5, 913 (1953).
2. A. M. Alekseev and N. A. Gusev, The Effect of Mineral Nutrition on the Water Regime of Plants [in Russian] (Izd. AN SSSR 1957).
3. P. S. Buntovaya et al. Collection of Scientific-Research Work of the Azovo-Chernomorsk Agricultural Institute [in Russian] (Novocherkassk, 1956) Vol. 14.
4. A. A. Nichiporovich, Light and Carbon Nutrition of Plants (Photosynthesis) [in Russian](Izd. AN SSSR, 1955).
5. A. A. Nichiporovich, Izvest. Akad. Nauk SSSR, Ser. Biol. 4, 3 (1952).
6. M. Ya. Shkol'nik, N. A. Makarova, et al., Proc. Botanical Inst. Akad. Nauk SSSR [in Russian] (Izd. AN SSSR, 1958) Series 4, Vol. 12.
7. M. Ya. Shkol'nik and T. V. Chirkova, Proc. Botanical Institute, Akad. Nauk SSSR [in Russian] Izd. AN SSSR, 1958) Series 4, Vol. 12.
8. E. N. Bazyrina and V. A. Chesnokov, Izvest. Akad. Nauk SSSR, Ser. 7, 10, 1317 (1931).

THE EFFECT OF SEVERING SOYBEAN LEAF VEINS UPON OUTFLOW OF ASSIMILATES*

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From the anatomical and cytological literature it is known that water reaches the plant leaf parenchyma cells not only directly along the xylem portion of the conducting tissues of the petiole, the central veins, and then into the net veinlets, but also by circuitous routes through anastamoses of the conducting pathways. The outflow of assimilates also takes place by the direct route, i.e., from the site of formation they flow into collecting cells, then to sieve tubes. Later, the assimilates flow into the phloem section of the conducting system of small and large veins, the main vein, the leaf petiole, and then into the stem. From these considerations it follows that in case of damage to the leaf blade the assimilate in the veins in the damaged part of the blade should not move. Interference with the outflow of assimilates usually leads to a disruption in metabolism and later to death of the tissue.

In practice, we frequently observe that when the leaf veins are damaged by wind, phytoparasites, etc., the leaf blade tissue usually still functions normally during the life of the leaf. Consequently, in many plants, we artificially damaged leaf blades by severing veins. The leaf blade, in these cases, was, in external appearance, no different from the remainder of the blade. This aroused doubt as to the correctness of the assertion that assimilates flow only by direct routes.

We decided to use labeled atoms (C^{14}) to trace the outflow of assimilates from isolated parts of the leaf after first blocking the path by severing the first-order veins. In this way we blocked movement along the primary pathway not only of assimilates, but of the water and the products of root activities, as well.

Soybean plants (variety Primorskaya 529) were used under field conditions. The leaves were left on the plants. In some leaves, the veins were severed at the base; in others, at the upper end. Cuts were made on one or both sides of the central vein of the leaf. In addition, during the administration of $C^{14}O_2$ as a gas curtain, leaves were partly covered with a strip of black paper. This was done so that photosynthesis was prevented in the darkened part of the leaf blade, and any movement of assimilates into it from the illuminated part of the leaf blade would be easily detected.

The veins were cut with a razor; immediately after, the leaf was placed in a chamber where it was fed $C^{14}O_2$. After the leaf was fed, the dark strips were removed. Fully mature leaves were used, where the growth process was either fully or nearly complete. In young, newly formed leaves, the tissue at the cut withered away in the chamber; thus, none of these leaves could be used in the experiment. The feeding experiments were carried up to flowering, and into the period of general flowering and legume formation.

The leaves were placed in a plastic chamber and subjected to a current of $C^{14}O_2$ for 10-15 min. The CO_2 concentration in the chamber was about 1% with 8-12 μC of C^{14}/g of CO_2. A leaf disc 15 mm in diameter was used for determining radioactivity.

Experiment 1. Soybean plants were fed $C^{14}O_2$ when flowering began on June 24. Leaf disc samples were taken four hours and three days after application of CO_2.

The experimental plants had seven leaves on the main stem. The third leaf from the top was fed CO_2.

The data on radioactivity at various loci on the leaf blade are shown in Fig. 1.

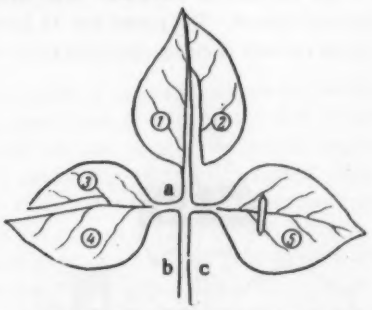

Fig. 1. Radioactivity of soybean leaf (counts/min on surface of leaf disc) on day $C^{14}O_2$ was fed to plant. Disc No. 1) 885; 2) 885; 3) 1135; 4) [no data given in original – Publisher.] 5) 354. Three days after feeding: Disc No. 1) 187; 2) 527; 3) 188; 4) 148; 5) 334.

* Student É. Ya. Kostetskii of the Far East State University assisted in this work.

The data in Fig. 1 show the following. In leaflet (a), in which part of the leaf blade (2) was separated from the right side of the main vein, the assimilates in the separated portion decreased 40% in three days. In the unseparated blade (1) assimilates decreased 79%. In leaflet (b), that part separated (3) from the main vein (the sample was taken where the veins were not severed), the assimilates during the same period decreased 84%, and in the undetached part (4) of the leaf blade, 86%. In leaflet (c), in which the main vein was severed, the assimilate outflow (5) was only 6%. These data indicate that when the primary pathway for movement of assimilates is damaged, the assimilates remain where they are formed, and they either do not move out during the initial days after damage or else move very slowly. When the veins are severed, turgor of the leaf tissues does not decrease; consequently, the water reaches this part of the leaf in necessary amounts by a circuitous route.

Experiment 2. Soybean plants on the day of the experiment, July 30, had seven leaves. Radioactive carbon dioxide was applied to leaves at the sixth node. Two leaflets, (a) and (b) (Fig. 2) were partly darkened with narrow strips of black paper during the feeding of $C^{14}O_2$. Leaf disc samples were taken after 24 hours on July 31.

The data obtained are depicted in Figure 2. In this experiment, just as in experiment 1, we see that the assimilates decrease several times slower in leaf blades separated from the main vein than in blades not separated from the main vein. The radioactivity data from the darkened parts of leaflets (a) and (b) show that the photosynthesis products practically are not translocated through the leaf either up or down, a fact which we have already noted [1]. The turgor of the leaves remained normal.

Experiment 3. Leaves of soybean were fed $C^{14}O_2$ at the time of full anthesis, on August 5. Leaf disc samples were taken on August 6. The plants had 11 leaves and the leaf at the seventh node on the main stem was fed $C^{14}O_2$.

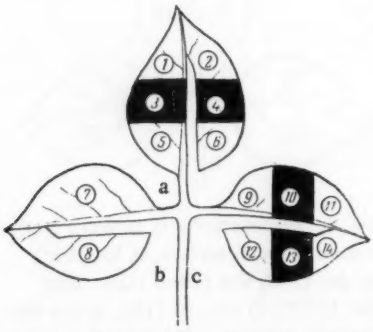

Fig. 2. Radioactivity of soybean leaf (counts/min on surface of leaf disc). Disc No. 1) 92; 2) 235; 3) 2; 4) 22; 5) 52; 6) 382; 7) 62; 8) 400; 9) 58; 10) 10; 11) 81; 12) 234; 13) 8; 14) 175.

Radioactivity was noted in the young leaves of nodes 10 and 11 of the main stem and in the apical leaves of all form branches. This indicates that assimilates from the experimental leaves were reaching the young developing leaves.

Leaf veins of the first order were separated from the main vein of the leaf blade just as in experiment 2. Leaflets (a) and (b) were partly darkened with strips of black paper. Figure 3 shows the leaf radioactivity data.

The data in Fig. 3 are analogous to that of Fig. 2. The flow of assimilates is retarded in those parts of the leaf blade where the first-order veins were severed from the main vein of the leaf. In those parts of the leaf blade where the lateral veins were not separated from the main vein, the outflow of assimilates was rapid. Turgor of all parts of the leaf blades both with and without severed leaf veins remained equally normal.

Experiment 4. In this experiment, the leaf blade was separated from the main vein of the leaf on two sides. In all three leaflets of one plant (Fig. 4) the leaf blade

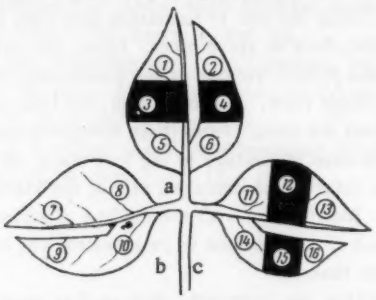

Fig. 3. Radioactivity of soybean leaf blades (counts/min on surface of leaf disc). Disc No. 1) 191; 2) 307; 3) 19; 4) 18; 5) 191; 6) 190; 7) 354; 8) 229; 9) 735; 10) 197; 11)147; 12) 11; 13) 156; 14) 170; 15) 9; 16) 311.

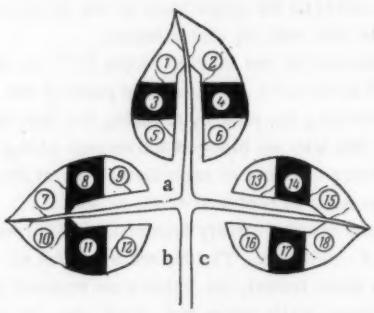

Fig. 4. Radioactivity of soybean leaf blades (counts/min on surface of leaf disc). Disc No. 1) 276; 2) 215; 3) 19; 4) 36; 5) 893; 6) 950; 7) 168; 8) 59; 9) 740; 10) 285; 11) 0; 12) 859; 13) 1092; 14) 0; 15) 169; 16) 1000; 17) 23; 18) 153.

TABLE 1. Radioactivity of Leaf Blades at Various Times (counts per min. on surface of leaf discs, d = 15 mm)

Location on leaf (see Fig. 6)	Sampling date		Percent assimilates in leaf on August 13
	Aug. 5	Aug. 13	
1	277	105	37.9
2	352	190	53.9
3	137	240	175.2
4	379	93	27.2
5	247	114	46.1
6	111	143	128.8
7	271	156	57.5
8	247	227	91.9

was severed from the base of the blade toward the apex. In another plant (Fig. 5) the blades were cut from the acropetal end toward the base. Both plants had about nine leaves on the main stem. The sixth leaf from the first plant and the fifth leaf of the second plant were exposed to $C^{14}O_2$. Just as in the three preceeding experiments, the veins were severed prior to exposure to $C^{14}O_2$. A portion of the leaf blade in both plants was darkened with black paper. The plants were exposed to $C^{14}O_2$ on July 30, and the leaf discs were taken two day later.

The data on the radioactivity of different parts of the leaf blades (Figs. 4 and 5) shows that the leaf assimilates are not cross translocated. The flow of assimilates from one part of the leaf to another takes place only by way of those veins which ramify in that part of the leaf. When the large veins which are joined to the main vein of the leaf are severed, the area served by these severed veins retains the assimilates and their efflux through other associated veins does not occur. The turgor of all leaf blades with severed veins remains undiminished.

Experiment 5. None of the three leaflets of the leaf at the eighth node of the soybean were partly severed from the main vein, but only the large veins were severed, as shown in Fig. 6, prior to exposure to $C^{14}O_2$. The plants

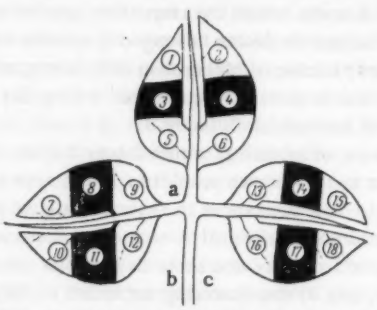

Fig. 5. Radioactivity of soybean leaf blades (counts/min on surface of leaf disc). Disc No. 1) 260; 2) 603; 3) 4; 4) 14; 5) 115; 6) 143; 7) 552; 8) 17; 9) 115; 10) 210; 11) 1; 12) 91; 13) 106; 14) 9; 15) 450; 16) 120; 17) 12; 18) 220.

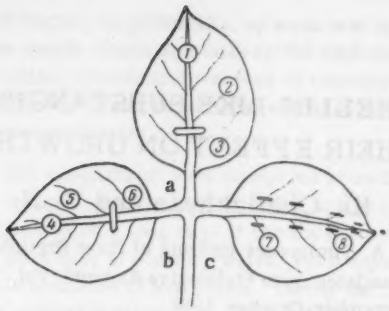

Fig. 6. Radioactivity of soybean leaf blades (counts/min on surface of leaf disc). See Table 1.

had two leaves on the main stem. The leaves were exposed to the radioactive carbon dioxide on August 4 and the leaf discs were taken on August 5 and 13.

The data are presented in Table 1.

The data of Table 1 show that on the second day after feeding, the concentration of assimilates decreased in the leaf blade more in places where the veins were undamaged than where the veins were severed, just as we noted in the previous five experiments.

Nine days after the leaves were exposed to $C^{14}O_2$, the picture of assimilate distribution in the leaf blades was the opposite of that which we observed on the second day after exposure to radiocarbon dioxide. It appeared that the concentration of assimilateswas greater where the veins were undamaged than where they were severed. These data indicate that when the primary direct route (veins) for the movement is damaged, the assimilates move out by circuitous routes, to all appearances through anastamoses in the conducting traces. However, the outflow of assimilates over the circuitous route apparently does not commence immediately after the veins are damaged.

SUMMARY

1. The products of photosynthesis are not circulated between isolated parts of a leaf blade with undamaged veins or even between cells of the assimilating tissue. The exit of assimilates from the mesophyll cells takes place only along the veins of the conducting system which abut the cells of the assimilatory tissue.

2. Each large or small leaf vein serves a certain part of the leaf blade from which assimilates are collected in a manner similar to the way in which water is collected from a basin by streams or rivers.

3. Water flows in the leaf cells not only by the direct route along the xylem strands serving a certain part of the leaf blade, but also through neighboring cells, presumably through anastomoses.

LITERATURE CITED

1. I. F. Belikov, Doklady Akad. Nauk SSSR 120, No. 4 (1958) [see English translation].

GIBBERELLIN-LIKE SUBSTANCES IN HIGHER PLANTS AND THEIR EFFECT ON GROWTH AND FLOWERING

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INTRODUCTION

Gibberellins were detected in secretions of the fungus Gibberella fugikori Saw., which represents a sexual stage of Fusarium moniliforme Sheld., and for many years the study of gibberellins and gibberellin-like substances was related to the functional and excretory activities of this fungus, and later to the pure gibberellin preparations obtained from culture solutions on which it was bred [1, 2]. Over a period of many years, three gibberellins were obtained, purified, and chemically identified from secretions of F. moniliforme: $A_1(C_{19}H_{24}O_5)$, $A_2(C_{19}H_{26}O_6)$, and $A_3(C_{19}H_{22}O_6)$ or gibberellinic acid, but comparatively recently a pure form of gibberellin was obtained $A_4(C_{19}H_{24}O_5)$ with properties similar to the first three [3].

Lately, facts have become known indicating a wider distribution of gibberellins among lower and higher plants. On one hand it was shown that gibberellin-like substances are contained in secretion of soil yeasts Torula pulcherrima [4] and that secretions of some other microorganisms exert a stimulatory action on the growth of higher plants. On the other hand, gibberellin-like substances were found in unripe seeds and fruits of wild cucumber (Echinocystis macrocarpa), avocado (Persea americana), Californian chestnut (Aesculus californica), common kidney bean (Phaseolous vulgaris), peas, corn lupin, tobacco, plum, apricot, and almond [5,6], in seedlings of normal and dwarf pea varieties, in pea seeds and runner bean seeds (Phaseolus mutiflorus) [7,9], in young rape racemes [10], and finally in unripe apple seeds [11].

In detecting the gibberellin-like substances, these authors used biological methods by which the comparative effects of gibberellins and extracts or diffusates on the growth of wheat leaf sections [7], oats [11], corn dwarf mutations [12], and dwarf form of peas [13] were tested. In further experiments the effect on plant flowering of gibberellin-like substances isolated from higher plants was also tested – the effect of substances isolated from the endosperm of wild cucumber (Echinocystic mirocarpa). Seedlings of biannual henbane (Hyoscyamus niger) flowered in the absence of low temperature, and plants of a long-day species Samolus parviflorus on a short-day environment [14], while under effects of substances isolated from unripe seeds of common beans (Phaseolus vulgaris) and bryophyllum plants (Bryophyllum crenatum) flowered in short-day environments [15]; the control plants remained in the vegetative growth stage.

All these investigations showed that gibberellin-like substances are contained in various organs of higher plants and are similar in their physiological activity to gibberellins found in secretions of the fusarium fungus. In later studies the presence of gibberellin A_1 had already been established in runner bean and common bean seeds [16, 17] and in fatty shoots of citrus [18]; also a new gibberellin A_5 was found in runner bean seeds [19].

The wide distribution of gibberellins and gibberellin-like substances in higher plants was most important in understanding the role of gibberellins in the processes of higher plants' flowering as it was conceived by the first author [20,21]. According to this theory, the flowering of all plants depends on the formation in the leaves of a flowering hormone complex – phlorigen, the composition of which includes two groups of substances: gibberellins, necessary for the formation and growth of stems, and substances necessary for flower formation, tentatively called anthesins. Both groups of substances are present in long-day species on a long day, in short-day species on a short day, in neutral species on both a long and short day environments under which the respective species readily flower. Failure to flower in long-day species on a short day occurs because of gibberellin deficiency, while failure to flower in short-day species on a long day occurs because of anthesin deficiency.

In order to substantiate this theory further, it was important to solve three problems: 1) the appearance of gibberellin-like substances in leaves of higher plants, 2) the determination of relative content of these substances in leaves of long-day and short-day species on a long and short day, and 3) the flowering induction of long-day species in short-day environments by gibberellin-like substances extracted from leaves of other species, particularly from leaves of short-day species cultivated on a long day.

METHODS AND EXPERIMENTAL CONDITIONS

Two experiments were conducted to solve the given problems. The first experiment was conducted during

winter and spring in a hothouse of the Institute of Plant Physiology, Academy of Sciences of the USSR from December 9, 1958 to April 4, 1959 and the second experiment was conducted during the summer in a greenhouse from July 20 to October 20, 1959.

Gibberellin-like substances were extracted from leaves of the following plants: long-day species – rudbeckia (Rudbeckia bicolor), Silvestris tobacco (Nicotiana silvestria), summer rape (Brassica napus v. oleifera): the short-day species – red leaf perilla (Perilla nankinensis) and Mamont tobacco (Nicotiana tabacum). In the winter experiment rudbeckia, redleaf perilla, and Mamont tobacco were used; in the summer experiment all the plants were used.

The plants were cultivated in environments of a long (L) and short (S) day; the long day consisted of the natural day to which additional incandescent electric light was added in winter and spring; the short 9-hour day was created in the hothouse by using a cloth chamber impervious to light, and in the greenhouse by rolling the plants on a wagon into a dark photoperiodic pavilion. After the beginning of bud and flower formation on a favorable day-length, leaf samples were taken for extraction from plants placed in differing light conditions. Leaves for sampling were cut from the middle tiers systematically during the whole period of experimentation. In the winter experiment samples were taken from plants sown in spring and cultivated during the growing season of 1958; in the summer experiment – from plants sown in the spring and cultivated during the growing season of 1959.

The extraction of substances was conducted as follows: a 20 g batch of fresh leaves (in the winter experiment) or a 30 g batch (in the summer experiment) were killed by alcohol vapors, placed between sheets of filter paper and squeezed by a hand press; for speedier drying of the paper a FÉN blower was used; for a more complete extraction of substances, the pressed leaf mass was saturated with acetone and squeezed for a second time in a press between other sheets of filter paper. Then the dried filter paper sheets were cut into pieces, placed in a column and washed with benzene until colorless after filtration, to remove lipoids; after washing and drying until the odor of benzene disappears, the pieces of filter paper were again placed in the column and washed drop by drop with acetone into an evaporating dish. The acetone was then evaporated and the dry residue was dissolved in 4 ml of distilled water.

The presence of gibberellin-like substances was first determined in the obtained extracts by the corn growth reaction [22]. This determination showed that there were gibberellin-like substances stimulating the growth of corn leaves in all the tested extracts.

The principal tests of the gibberellin-like substance activity of leaf extracts were conducted on rudbeckia plants [23]. Large rosette rudbeckia plants placed on a short 9-hour day were used for both experiments. The plants were treated by the drop method: one drop of the tested extract, or gibberellin, or water was applied daily on the rosette center and later on the stem summit of each plant. Gibberellin was used in concentrations of 0.02% in the winter experiment and of 0.005% in the summer experiment.

The experiments were conducted according to this plan with all species: 1) gibberellin; 2) extract from plant leaves on a long day; 3) extract from plant leaves on a short day; 4) control - water. Each experimental sample consisted of four plants. During the entire experiment rudbeckia plants were in environments of a short 9-hour day. Treatment of plants by extracts lasted 90 days in the winter experiment (from December 9, 1958 to March 8, 1959), and in the second summer experiment 50 days (from July 20 to September 10, 1959).

RESULTS

In the first experiments, conducted during winter and spring in less favorable environments than the second experiment, which was carried out during summer in the greenhouse, the rudbeckia plants developed more slowly despite the additional electric illumination than in the summer growth period. Therefore the plant's reaction to the activity of studied extracts in the winter experiments was weaker than in summer. Moreover, the extracts used in the winter experiment were weaker since the batch of initial material was smaller than in the summer experiment. However, both experiments showed similar results.

As usual, a quick reaction in the winter experiment was shown by plants treated with gibberellin: after 13 days the plants formed shoots, then grew high stems and began to bud and flower. The control plants remained in rosette stage up to the end of the experiment. A different picture was presented by the experimental plants, as seen from Table 1.

As a result of treatment by leaf extracts of rudbeckia, Mamont tobacco, and perilla cultivated on a long day, all the plants formed shoots, but only individual specimens formed buds and flowers, and only specimens treated by extracts from rudbeckia and Mamont tobacco leaves reached the flowering stage. In variants treated by extracts from leaf plants cultivated on short-day shoots, flowers and buds were produced by one plant and at a much slower tempo, while in the perilla, variant plants remained in the stage of shoots (Figs. 1 and 2).

Table 1 shows that all the tested extracts contain gibberellin-like substances and that an irregularity of reaction is observed by rudbeckia plants being treated by their extracts: in each variant one or two plants reacted most powerfully to the treatment. It was found that just these plants differed by somewhat larger rosette dimensions at the beginning of the experiment, from which it can be deduced that the most effective action of extracts was manifested on plants characterized by a high growth intensity [24].

TABLE 1. Effect of Gibberellin-like Substances from Plant Leaves on Rudbeckia Growth and Flowering (Winter experiment, 1958-1959)

Experimental variants	Day Length	Shoots	Bud formation	Flowering	Stem growth* in cm on April 4	
					height	thickness
Gibberellin 0.02%	–	Dec. 22	Feb. 16	Mar. 5*	101	0.7
Extract from rudbeckia leaves	L	Dec. 23	Mar. 10*	Apr. 10*	9	1.8
"	S	Dec. 27*	Mar. 12*	May 4*	2	1.8
Extract from Mamont tobacco leaves	L	Jan. 6	Feb. 12 Apr. 30	Mar. 8*	48	1.7
"	S	Jan. 20*	Mar. 7*	Mar. 26*	30	2.0
Extract from perilla leaves	L	Jan. 6	Apr. 12	–	4	1.6
"	S	Dec. 31*	–	–	2	1.7
Control, water	–	–	–	–	Rosette	

* Data for one plant.

TABLE 2. Effect of Gibberellin-like Substances from Plant Leaves on Rudbeckia Growth and Development (Summer experiment of 1959)

Experimental variants	Day length	Shoots	Budding	Flowering	Stem growth in cm on Oct. 10		Raw weight of one plant in g on Oct. 20
					height	thickness	
Gibberellin 0.005%	–	July 27	Aug. 17	Aug. 29	148	1.2	149
Rudbeckia leaf extract	L	Aug. 4	Sept. 4	Oct. 10*	28	2.1	213
"	S	Sept. 10	–	–	4	1.9	170
Silvestris tobacco leaf extract	L	Aug. 3	Aug. 20	Sept. 17	35	2.1	210
"	S	–	–	–	Rosette		187
Summer rape leaf extract	L	Aug. 16	Aug. 25	Sept. 15	39	2.5	179
"	S	Aug. 18	Sept. 3	Oct. 7**	9	2.1	142
Mamont tobacco leaf extract	L	Aug. 2	Aug. 13	Sept. 4	71	1.8	223
"	S	Aug. 4	Aug. 28**	Sept. 2**	17	2.5	202
Perilla leaf extract	L	Sept. 6	–	–	2	2.0	189
"	S	–	–	–	Rosette		178
Control, water	–	–	–	–	Rosette		119

*Data for one plant.
**Data for two plants.

In the summer experiment the reaction of rudbeckia plants to extract activity was much more regular and distinct, as seen from Table 2.

All the plants speedily produced shoots, buds and flowers when gibberellin was used; all control plants remained in the rosette stage until the end of the experiments.

All plants treated by extracts from rudbeckia, Silvestris tobacco, summer rape and Mamont tobacco leaves, placed on a long day formed shoots, buds and flowers, although in the case of rudbeckia only one plant flowered; in the variant with perilla extract, plants remained in the stage of shoots. When plants were treated by extracts

Fig. 1. Effect of rudbeckia leaves extract on growth and flowering of rudbeckia. 1) Extract from plant leaves on a long day; 2) extract from plant leaves on a short day; 3) control, water (photo April 23, 1959)

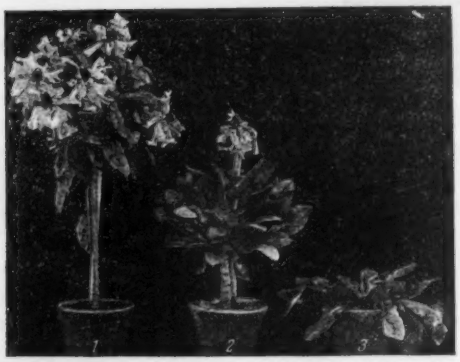

Fig. 2. Effect of Mamont tobacco leaf extracts on growth and flowering of rudbeckia. 1) extract from plant leaves on a long day; 2) extract from plant leaves on a short day; 3) control, water (photo April 23, 1959)

from rudbeckia, summer rape and Mamont tobacco leaves placed on a short day, all the plants produced shoots, but buds and flowers appeared only in variants with extracts from summer rape and Mamont tobacco; plants treated by extracts from Silvestris tobacco and perilla leaves on a short day did not produce shoots (Fig. 3 and 4).

Results obtained in the summer experiment indicate even to a greater degree that leaf extracts of different higher plants contain gibberellin-like substances which produce an effect on the growth and flowering of rudbeckia rosette plants in short-day environments. Therefore, turning to the problems stated at the beginning of this article, it can be assumed that the first problem, the existence of gibberllin-like substances in leaves of higher plants is decidedly solved.

The second problem of determining the relative content of gibberellin-like substances in leaves of short-day and long-day species on a short and long day also reaches a known solution. In the experiment with wheat sprouts as well as in both experiments with rudbeckia rosettes, the content of gibberellin-like substances in extracts from plant leaves of long-day as well as short-day species is always higher on a long day than in leaf extracts on a short day.

Considering the third problem, the possibility of inducing flowering of long-day species in environments of a short day with help from gibberellin-like substances extracted from leaves of other species, and particularly from leaves of short-day species on a long day, it can be assumed that results obtained from the experiments also yield a positive response. If extracts are taken for comparison from leaves of two tobacco species – the long-day Silvestris and the short-day Mamont, then the following picture is obtained (Fig. 5 and 6).

As seen from Fig. 5, the stem growth and flowering of rudbeckia came as a result of the effect of the Silvestris tobacco leaf extract which was flowering and cultivated on a long day. It could be assumed that in this case the flowering of rudbeckia occured at the expense of those substances which form only in leaves of a flowering plant. Such an assumption could be supported by many facts obtained in experiments with graftings and indicating the possibility of flowering by vegetating

Fig. 3. Effect of extracts from Silvestris tobacco leaves on rudbeckia growth and flowering. 1) gibberellin preparation (0.01%); 2) extract from plant leaves on a long day; 3) extract from plant leaves on a short day; 4) control, water (photo September 18, 1959)

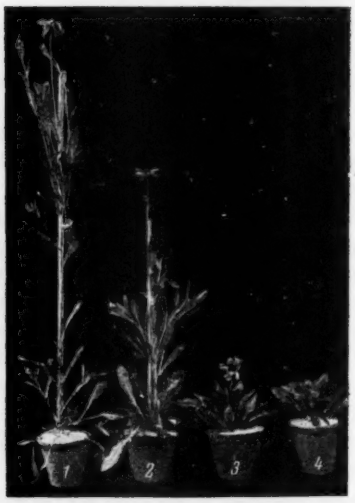

Fig. 4. Effect of extracts from Mamont tobacco leaves on rudbeckia growth and flowering. 1) Gibberellin preparation (0.01%); 2) extract from plant leaves on a long day; 3) extract from plant leaves on a short day; 4) control, water (photo September 18, 1959)

plants at the expense of substances which form only in leaves of a flowering plant on which they were grafted [25-26].

However, the comparison in Fig. 6 clearly indicates that such an assumption would be incorrect since in this case the stem growth and flowering of rudbeckia takes place by the effect of extracts obtained from leaves of short-day Mamont tobacco placed in environments of a long day and remaining in a vegetative state.

Thus, the deduction should be made that the stem growth and flowering of rudbeckia came at the expense of gibberellin-like substances present in extracts of Mamont tobacco leaves on a long day and at the expense of anthesins present in rudbeckia leaves on a short day.

DISCUSSION

The detection of gibberellin-like substances in this work was performed by extracting substances with acetone and direct tests of their activity on plants without chromatography and separation of individual fractions of active substances, as had been done by the majority of authors cited above [5-11, 16-19]. The absence of chromatographic data in this work, of course, does not permit any conjecture as to what degree the found gibberellin-like substances contain gibberellins or are close to them in their adsorption properties, and what the composition is of these gibberellin-like substances, as judged by the histograms. However, the specific reaction of rudbeckia stem growth and rosette flowering in short-day environments, which is manifested only on gibberellin activity, and which does not appear on activity of auxins and vitamins [23, 27], affords a complete basis for stating the presence of gibberellin-like substances in the obtained extracts, even though in combination with many other compounds extracted by acetone. There is no doubt that the next problem in this direction is a detailed chromatographic study of individual components of gibberellin-like substances extracted from leaves and other green organs, and an approach to their chemical identification.

Very recently there were attempts to study the presence of gibberellin-like substances in leaves of higher plants and changes in their composition related to photoperiodism and the transition of plants from vegetative growth to the formation of buds and flowers.

In Harada and Nitsch [28] experiments with rudbeckia (Rudbeckia speciosa) and two chrysanthemum varieties (Chrysanthemum morifolium) and in Lang [29] experiments with annual henbane (Hyoscyomus niger), extracts were taken from leaves of vegetating and induced plants and were studied chromatographically and biologically – the reaction was added growth of oat leaves and dwarf mutants of corn. The authors came to a common conclusion that,on induction of plants to flowering, changes occur in the composition of gibberellin-like substances of their leaves.

Most probably changes occur in the composition of gibberellin-like substances when plants are induced to

Fig. 5. Rudbeckia flowering in short day environments effected by plant leaf extracts from long-day Silvestris tobacco cultivated on a long day. 1) Silvestris tobacco plant from leaves of which the extract was taken; 2) rudbeckia plant treated by the extract; 3) control rudbeckia plant (photo September 18, 1959)

Fig. 6. Rudbeckia flowering in short-day environments effected by extracts from plant leaves of short-day Mamont tobacco cultivated on a long day. 1) Mamont tobacco plant from the leaves of which the extract was taken; 2) rudbeckia plant which was treated by this extract; 3) rudbeckia control plant (photo September 18, 1959)

flower by long and short photoperiods, as the data show [28,29]. However, as the experiments described here show, the changes in the total amount of gibberellin-like substances and their activity in plant leaves are not related to the transition of plants to flowering but directly to the effect of a long day.

It should also be remembered that the content of native auxins in leaves and stem tops of all plants, irrespective of their photoperiodic reaction, is always larger on a long day than on a short one [30]. And insofar as a close interrelation between auxins and gibberellins exists [8,27,31,33] it seems very probable that the greater the content of auxins on a long day, the content of gibberellins and gibberellin-like substances is correspondingly greater, and this determines the greater growth intensity of plants in long day environments.

In connection with the discovery of gibberellins and gibberellin-like substances the question arises of their role in the process of plant flowering. As indicated by the data of numerous authors [1,2,20,21,23] there is no doubt that gibberellins are important to most long-day species. Results depicted in this experimental study indicate that gibberellin-like substances extracted from leaves of vegetating and flowering plants also cause flowering of long-day species in environments of a short day, and confirm the assumption that in these environments the long-day species show a gibberellin deficiency while substances necessary for flower formation tentatively called anthesins are present.

SUMMARY

Gibberellin-like substances have been detected in the leaves of long-day plants such as rudbeckia (Rudbeckia bicolor), tobacco (Nicotiana silvestris) and rape (Brassica napus v. oleifera), as well as in short day plants Perilla nankinensis and Nicotiana tabacum. The substances were detected by extraction with acetone and a subsequent test of their action by observing the specific reaction on stem growth and flowering of rudbeckia under short day conditions. The results obtained, as well as those of other authors who found gibberellins and gibberellin-like substances in seeds and fruits, shoots and seedlings, inflorescences, and leaves, indicate that these substances are widely distributed in the tissues and organs of higher plants.

The total amount of gibberellin-like substances in plants depends on the day length. Irrespective of the nature of the photoperiodic reaction, it was found to be higher under long-day than under short-day conditions in all plants studied. These changes in the content of gibberellin-like substances concur with the previously discovered changes in the auxin content and with the enhancement of plant growth with increase of day length. Transition of the plants from vegetative growth to flowering does not involve a change in the content of gibberellin-like substances.

Gibberellin-like substances encountered in the leaves of higher plants affect stem growth and flowering in rudbeckia in exactly the same way as do pure gibberellin preparations. Stem growth and flowering of the vegetating rosettes of the long-day rudbeckia plant under short-day conditions induced by leaf extracts from the short-day plant Nicotiana tabacum which grew and vegetated under long-day conditions seem to be of special importance. This confirms the suggestion previously made that flowering of plants commences in the presence of two groups of substances in the leaves,

viz. gibberellins (in the tobacco extract) and substances influencing flower formation and tentatively called anthesins (in the rudbeckia tissues).

LITERATURE CITED

1. B. B. Stowe and T. Yamaki, Annual Rev. Physiol. 8, 181 (1957).
2. B. B. Stowe and T. Yamaki, Science 129, 807 (1959).
3. N. Takahashi, Y. Seta, H. Kitamura, and J. Sumiki, Bull. Agric. Chem. Japan 21, 396 (1957).
4. N. A. Krasil'nikov, M. Kh. Chailakhyan, L. V. Aseeva, and L. P. Khlopenkova, Doklady Akad. Nauk SSSR 123, 6, 1123 (1958).*
5. C. A. West and B. O. Phinney, Plant Physiol, Suppl. 31, 20 (1956).
6. B. O. Phinney, C. A. West, M. Ritzel, and P. M. Neely, Proc. Nat. Acad. Sci. USA 43, 5, 398 (1957).
7. M. Radley, Nature 178, 4541, 1070 (1956).
8. M. Radley, Ann. Bot. 22, 87, 297 (1958)
9. A. J. McComb and D. J. Carr, Nature 181, 4622 (1958).
10. F. Lona, Ateneo parmense 28, 111 (1957).
11. J. P. Nitsch, Bull. Soc. Bot. France 105, 9, 479 (1958).
12. B. O. Phynney, Proc. Nat. Acad. Sci. USA 42, 185, (1956).
13. P. W. Brian and H. G. Hemming, Physiol. Plantarum 8, 12 (1955).
14. A. Lang, J. A. Sandoval and A. Berri, Proc. Nat. Acad. Sci. USA 43, 960 (1957).
15. R. Bönsow, J. Penner, and R. Harder, Naturwissenschaften 45, 2, (1958).
16. J. MacMillan and P. J. Suter, Naturwissenschaften, 45, 2, 46 (1958).
17. C. A. West and K. H. Murashige, Plant Physiol. 33, Suppl. 38 (1958).
18. A. Kawarada and Y. Sumiki, Bull. Agric. Chem. Soc. Japan 23, 343 (1959).
19. J. MacMillan, J. C. Seaton, and P. J. Suter, Proc. Chem. Soc. 325 (1959).
20. M. Kh. Chailakhyan, Fiziol. Rastenii 5, 541 (1958).*
21. M. Kh. Chailakhyan, Biolog. Zbl. 77, 6, 641 (1958).
22. A. N. Boyanrin and M. L Dmitrieva, Fiziol. Rastenii 6, 741 (1959).*
23. M. Kh. Chailakhyan, Bot. Zhur. 43, 927 (1958).
24. M. Kh. Chailakhyan and V. N. Lozhnikova, Doklady Akad. Nauk SSSR 128, 6, 1309 (1959).*
25. M. Kh. Chailakhyan, Hormonal Theory of Plant Development [in Russian] (Izd. AN SSSR, 1937).
26. A. Lang, Annual. Rev. Plant Physiol. 3, 265 (1952).
27. M. Kh. Chailakhyan and L. P. Khlopenkova, Doklady Akad. Nauk SSSR 129, 2. 454 (1959).*
28. H. Harada and L P. Nitsch, Plant Physiol. 34, 409, (1959).
29. A. Zang, Planta 54, 5, 498 (1960).
30. M. Kh. Chailakhyan and L. P. Zhdanova, Doklady Akad. Nauk SSSR 19, 6, 1-2, 107 (1938).
31. T. Hayashi and Y. Muracami, Agric. Chem. Soc. Japan 28, 543 (1954).
32. A. J. Veitos and W. Meudt, Nature 180, 284 (1957).
33. P. W. Brian, Biol. Rev., 37 (1959).

* See English translation.

THE ROLE OF LEAVES AND BUDS IN THE FORMATION OF ROOTS IN STEM CUTTINGS

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In plants, the processes of growth and development are realized under conditions of continuous interrelationship of the separate organs. This is usually defined as correlation. Michurin [1] showed in his studies that correlations are inherited, and he made use of them in the creation of new types of fruit.

The extensive literature of recent years on the subject of unity is mainly related to animal organisms, while summarizing data relating to unity in a plant organism were almost entirely lacking. The problem of unity in a plant organism in the process of growth and development during ontogenesis was integrated, in the light of contemporary data, in the recent publications of Chailakhyan [2,3]. The author points out that the interrelationship of organs in plants is particularly sharply defined during their transition from vegetative growth to generative development.

While a plant organism represents a united whole, separate parts of it possess a distinctly expressed capacity for independent existence, even after their separation from the mother plant. Unlike animals, every organ or part of a plant is capable, under certain conditions, of developing into an independent organism when separated from the whole mother plant. For instance, twigs, roots, or leaves, when separated from the mother plant, are capable of becoming independent organisms. This property of the plant is made use of in cuttings.

The separated part of a plant is also subject to correlative influences. Thus, for instance, root formation in a stem cutting depends on the existing ratio of buds and leaves, as well as on the character of the relationship between the upper and lower portions of the cuttings. There are indications in the literature that leaves and buds play an important part in the formation of roots in cuttings. Thus, Went [4] and Molish [5] made the observation that in some woody cuttings deprived of buds, the root formation proceeds either weakly, or not at all. Van der Lek [6], in his work with cuttings of various plants, and Loeb [7], in experiments with stem cuttings of Bryophyllum, came to the conclusion that the presence of leaves and buds facilitates the formation of roots in cuttings. A close relationship between root formation and the presence of leaves and buds was shown in the experiments of Zimmerman and Hitchcock [8], Mirimanyan [9], and Buzinova [10]. Ivanov [11] points out the close interdependence of leaves and roots, and grants the existence of a mechanism linking the activity of the roots to the oxidation-reduction regimen of the leaves. Kruzhilin and Shvedskaya [12] showed also that the formation and growth of the root system and the differentiation of vernalized buds in beet, carrot, and cabbage takes place only in the presence of leaves.

The role of leaves in root formation of cuttings was demonstrated by Overbeek and Gregory [13] in an original way. They used two types of roses, a red, the cuttings of which take root only when treated with a growth-stimulator, and a white, incapable of propagation by cuttings even with the application of a growth-stimulant. By grafting the red rose onto the white, they obtained rooting of the latter, while the removal of the leaves from the graft deprived the white rose cutting of this rooting ability. Evidently, there took place a flow of some substances from the leaves of the graft into the stock, which caused its twigs to take root.

It should be pointed out that leaves are not obligatory for all cuttings, depending upon physiological condition. Woody cuttings, rich in nutritive substances, do not need the leaves. On the other hand, green cuttings, which are poor in nutritive substances, require leaves for assimilation; but a large number of leaves on a cutting causes extensive transpiration, which may cause the cuttings to dry out before any roots are formed.

The objective of our present investigation consisted of finding out the optimal number of leaves to be left on a cutting for optimal root formation, as well as the effect of the ratio stem-buds-leaves on the regeneration process in stem cuttings. The rooting of all of the cuttings was conducted under conditions of dispersed light and darkness. The specimens consisted of green cuttings of the Polevka cherry and the Goliath black currant.

The experimental setup was as follows: 1) with leaves and buds; 2) with leaves but without buds; 3) without leaves but with buds; 4) without leaves or buds; 5) with one leaf; 6) with two leaves; and 7) with three leaves.

TABLE 1. The Role of Leaves in the Rooting of Polevka Cherry Cuttings

Number of leaves in a cutting	Treated cuttings	Percent of rooted	Remarks
3	IBA	45	Roots of medium size, length 6-8 cm, calluses large
	Water	25	Roots small, of 3-4 cm length
	IBA	100	Roots good, of 12-15 cm length, a large number of supplementary roots
	Water	26	Roots small
	IBA	60	Roots of 5-6 cm length, of smaller size as compared with the cuttings with three leaves, with IBA treatment
	Water	20	Roots small
Without leaves	IBA	0	Perished
	Water	0	Perished

One half of each group was treated with stimulators.

All of the black currant cuttings with various numbers of leaves took root within 12-18 days when exposed to light. Root formation was observed in cuttings with three leaves, followed by those with one or two leaves. Treatment with the potassium salt of heteroauxin accelerated the process of root formation in cuttings with a various number of leaves. To begin with, the cuttings with one leaf rooted more slowly than the others but gradually did take root.

The currant cuttings, when kept in the dark, did not form roots even after a period of 11 days, but did not lose their leaves. After a total of 25-28 days in the dark, the cuttings lost the leaves and half of the former perished, the remaining cuttings perishing soon thereafter in spite of the fact that individual cuttings had formed from one to two weak roots.

The Polevka cherry is a readily rooting plant, but its cuttings take root considerably more slowly and with less ease than the black currant cuttings. After 11 days in a lighted hot bed, the cherry cuttings showed the formation of calluses, the latter being larger than cuttings treated with indolbutyric acid (IBA), as compared with the corresponding controls. At that time, the cuttings which were kept in the dark did not form calluses in any of the series, and subsequently they perished. At the beginning of October, the cherry cuttings, which were kept in a lighted hot bed, were dug up, and the percent of rooted cuttings, as well as the character of root growth, was established (Table 1).

The conducted experiments showed that the green cuttings of cherry and currant thrive best in light when two or even three leaves are present, providing that in the currant, half of the leaf blade is cut off.

In the currant, cuttings with leaves and without buds take root as do cuttings with leaves and buds. As for cherry cuttings with three leaves and no buds, only 45% took root (after treatment with IBA), while the roots formed slowly and in small numbers, averaging 1-3 roots per cutting. When dug up in October, these cuttings had quite young roots with smalls amounts of supplementary rootlets. Cuttings with two leaves and buds took root 100% (with IBA treatment), with the formation of good roots up to 10 cm in length, with large calluses, and with a large number of old supplementary roots. The presence of supplementary darkened roots indicates that they were formed long ago (Fig. 1).

The series without leaves, but with buds, and without leaves or buds, did not form any roots and gradually perished. Thus, in green cuttings, the decisive role in root formation is played by the leaves.

Evidently, not all plants require, to the same degree, the presence of buds for root formation. This probably depends on the correlative connections between the mother plant and the twig before it is cut, as well as between the leaves and buds on the cutting as such. For instance, in green cuttings of cherry, treatment with IBA does not substitute for the buds in respect to the process of root formation. But in green cuttings of the Japanese spindle tree, with leaves, the growth stimulating substance is a complete substitute for the absent buds (Table 2). The cuttings of the Japanese spindle tree with leaves and buds, as well as without buds, took root 100%, with the formation of a well developed root bunch when treated with the potassium salt of heteroauxin. When treatment with a growth stimulator was omitted, 70% of the cuttings with leaves and buds took root, as against 20% without buds, the root system in the latter case being poorly developed. Perhaps, in the cuttings of the Japanese spindle tree deprived of buds, the synthetic heteroauxin is able to substitute for the natural auxin, which flows from the buds to the area of root formation.

In a repeat experiment on the role of leaves, light, and darkness in the rooting of currant cuttings, the latter were not kept in the dark up to the end of the experiment. After 18 days, when the leaf containing cuttings in the

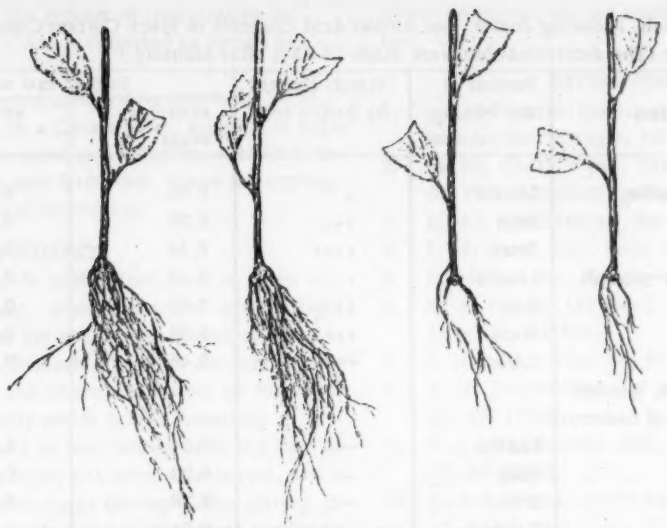

Fig. 1. Green cutting of Polevka cherry. On the left, cuttings with bud; on the right, without buds.

TABLE 2. The Role of Buds in the Rooting of Stem Cuttings, with Leaves, of the Japanese Spindle Tree (the cuttings were planted August 2)*

Experimental series	September 4			September 19		
	Cuttings %		Degree of root bunch development	Cuttings %		Degree of root bunch development
	with calluses	rooted		with calluses	rooted	
Control cuttings with buds	100	—	—	30	70	+ + +
Control cuttings without buds	90	—	—	80	20	+
Cuttings with buds, treated with the potassium salt of heteroauxin	—	100	+ + + + +	—	100	+ + + + +
Cuttings without buds, treated with the potassium salt of heteroauxin	—	100	+ + + + +	—	100	+ + + + +

*The experiments on the Japanese spindle tree were conducted in association with N. S. Klyushkina.

lighted hot bed began to take root and the cuttings in the darkened hot bed remained without changes, the darkening was discontinued, and the cuttings therein also took root without exception. All of the green cuttings deprived of leaves perished, irrespective of whether they were exposed to diffuse light or kept in the dark. A partial rooting of 30% was observed only in those currant cuttings deprived of leaves which were exposed to white luminiscent light, but heteroauxin here proved ineffective. Similar observations were made on phaseolus cuttings [14]. The comparatively more active formation of roots in cuttings deprived of leaves and exposed to luminescent light may evidently be explained by the fact that these rays slightly activate the process of photosynthesis in the upper end of the cutting's stem.

The histochemical test for starch showed that within 25 days following exposure to diffused light of control currant cuttings with leaves which have taken root, there was a large accumulation of starch, while in cuttings treated with heteroauxin, all of the starch was hydrolyzed due to abundant root formation. The cuttings deprived of leaves, as well as those with leaves but kept in the dark, also contained practically no starch.

The quantitative determination of reducing sugars and amino acids may also characterize, to a certain ex-

TABLE 3. The Starch, Reducing Sugars, and Amino Acid Contents in Black Currant Cuttings, With and Without Leaves (The determinations were made 25 days after planting.)

Experimental series	Portion of the cutting	Starch content by iodine test	In % of raw weight	
			reducing sugar	amino acids
Cuttings before planting	Leaves	+	0.38	0.08
	Buds	+++	0.36	0.05
	Stem	++++	0.34	0.02
Cuttings with leaves-controls	Leaves	+	0.58	0.14
	Buds	++	0.62	0.20
	Stem	+++	0.36	0.06
	Callus	+++	0.48	0.19
Cuttings with leaves, treated with potassium salt of hetero-auxin	Leaves	–	0.64	1.11
	Buds	–	0.28	0.12
	Stem	–	0.26	0.04
	Callus	–	0.54	0.14
Cuttings without leaves-controls	Buds	–	0.18	0.05
	Stem	+	0.24	0.03
Cuttings without leaves, treated with potassium salt of heteroauxin	Buds	–	0.14	0.02
	Stem	–	0.1	0.03

tent, the physiological condition of the cuttings which were exposed to diffuse light (Table 3).

The reducing sugars and amino acids are found mainly in control cuttings with leaves which have taken root, as is the case with starch. A high sugar and starch content is found in the callus, the newly formed organ. This fact indicates a transport of nutritive substances to the site of root formation. Data on the amino acid contents in the various series are an indication that carbohydrates are the first to be utilized in respiration and roots formation.

It is difficult to say why the absence of buds in green currant cuttings does not affect root formation, as in the cherry. The analysis of the plastic substances explains it only partially (Table 4). In the cherry, as can be seen from Table 4, the maximum amount of reducing substances is contained in the buds. The leaf blade of the cherry is small, while the bud is comparatively large, which is the reason that in the cherry the presence of buds exerts an influence on the rooting process. In the currant, the reducing substances are distributed more evenly in all parts of the cutting. The leaf blade is quite large in the currant, while the buds are quite poorly developed.

We also studied the question of whether sugar, nitrogen and growth stimulants are capable of substituting for the leaves in a green cutting. For this purpose, cuttings with and without leaves received glucose, ammonium sulfate, and IBA, in various ratios. In cherry cuttings with leaves, these substances enhanced the process of root formation. Cuttings, deprived of leaves, of the series without treatment and of the series glucose + ammonium sulfate, took root to an extent of 20%, and upon the addition of IBA, 25%. But in these series the roots of the cuttings developed feebly: In one cutting, there developed only one or two small roots. On the other hand, the cuttings of the last series with leaves took root to an extent of 85%, developing long roots with a large number of auxiliary roots. While sugar, nitrogen, and IBA, in the cuttings of the Polevka cherry, enhance the process of root formation, they seem unable to substitute for the function of the leaves.

Thus, the leaves and buds play an important role in root formation by stem cuttings. It is possible to render the cuttings to a certain extent more plastic and physio-

TABLE 4. The Reducing Sugar and Amino Acid Content in Various Parts of Cherry and Currant Cuttings (in % of Raw Weight)

Portion of the cutting	Cherry		Currant	
	reducing sugars	amino acids	reducing sugars.	amino acids
Leaves	0.76	0.09	0.38	0.08
Buds	1.46	0.06	0.36	0.05
Stem	0.42	0.03	0.34	0.02

logically prepared for the process of regeneration by means of various agents and a variation in correlative interdependence.

Different results were obtained by Overbeek et al. [15], who showed that in a Chinese rose, arginine or sugar and ammonium sulfate serve as a complete substitute for the leaves needed for root formation, where the cuttings were treated with growth stimulants.

SUMMARY

1. The decisive role in root formation in green cuttings belongs to the leaf. When exposed to diffused light, cuttings without leaves are unable to regenerate roots. Darkness inhibits root formation in green cuttings of cherry and black currant, and where weak roots are formed in the dark, they invariably perish from exhaustion.

2. The dependence of root formation at the base of a cutting from the presence and number of leaves, and of their photosynthetic activity at the top of the cutting, indicates a close relationship between the upper and lower parts of the cutting.

3. Green cherry cuttings with leaves, but without buds, take root to a much lesser degree than similar cuttings with buds. In green cuttings of black currant with leaves, the absence of buds does not affect root formation. In cuttings of the Japanese spindle tree with leaves, but without buds, the potassium salt of heteroauxin substitutes the function of the buds in respect to root formation. It may be assumed that in this instance the stimulator of growth replaces the natural auxin, which flows from the buds to the site of root formation.

4. The high content of sugar and starch in the callus of the black currant cuttings indicates a transport of nutritive substances via the cutting to the site of root formation.

LITERATURE CITED

1. I. V. Michurin, Results of Sixty Years' Work [in Russian] (Sel'khozgiz, 1934).
2. M. Kh. Chailakhyan, The Unity of the Organism in the Plant Kingdom [in Russian] (Izd. Arm SSR, 1955).
3. M. Kh. Chailakhyan, Bot. Zhur. 41, 487 (1956).
4. F. W. Went, Proc. Sect. of Sciences 32, 35 (1929).
5. H. Molish, Ber. d. Deutsch. Bot. Ges. 53, 575 (1935).
6. H. A. van der Lek, Med. van de Landbouw. Wageningen 28, 3 (1925).
7. J. Loeb, Bot. Gaz. 63, 25 (1917).
8. P. W. Zimmerman and A. E. Hitchcock, Am. J. Bot. 15, 626 (1928).
9. V. A. Mirimanyan, Bull. po Vlazhnym Subtropikam 10, 60 (1943).
10. A. I. Businova, Trudy Instituta Lesa Akad. Nauk SSSR 11, 253 (1953).
11. S. M. Ivanov, Doklady Akad. Nauk SSSR 88, 23, 567 (1953).
12. A. S. Krushilin and Z. M. Shvedskaya, Doklady Akad. Nauk SSSR 124, 7, 1353 (1959). *
13. J. V. Overbeek and L. E. Gregory, Am. J. Bot. 32, 336 (1945).
14. R. Kh. Turetskaya, Doklady Akad. Nauk SSSR 76, No. 37 (1951).
15. J. V. Overbeek, S. A. Gordon, and L. E. Gregory, Am. J. Bot. 33, 100 (1946).

* See English translation.

PHYSIOLOGICAL AND BIOCHEMICAL CHARACTERISTICS OF POLLEN AND PISTILLATE TISSUES OF CERTAIN MEMBERS OF THE ROSACEAE

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There is little published information on the physiological and biochemical characteristics of angiosperm male and female gametophytes. In the main, it consists of descriptions of differences between pollen and pistillate tissues with respect to enzyme activity [1-3], oxidative metabolism [4-8], levels of nutrient reserves [3], pigments [9, 10, and others], vitamins [11, 12], growth stimulators, etc.

Only in recent studies [3, 5, and others] has there been a shift from enumeration of these differences to elucidation of their role in fertilization (mainly in the progamic stage).

It should be noted that most of the studies mentioned above on the physiology of fertilization in angiosperms were carried out with annuals; perennials have received very little attention in this respect. We therefore decided to investigate certain physicochemical and physiological aspects of sexual dimorphism during fertilization in the most widespread orchard species of the family Rosaceae, subfamilies Prunoideae (Cerasus vulgaris, Certsus tomentosa, Cerasus Besseyi, Prunus triflora, Armenica vulgaris, Persica vulgaris, Amygdalus gibridica, Prunus divaricata, Prunus domestica, Prunus spinosa) and Pomoideae (Malus domestica, Pyrus communis).

We were interested, first, in the oxidation-reduction properties of the sexual elements, i. e., those properties which, in our view, most highly characterize sexual dimorphism [13], and, secondly, in the reserve nutrients which supply energy and support synthesis during the approximation of the sexual elements in fertilization.

The following physiological criteria were employed: 1) plant lifetime acidity, pH_n; 2) character of the oxidizing properties of cells and tissues, rH_2; 3) extent of the oxidizing-reducing (OR) potential, Eh; 4) oxidative enzyme (phenolase, peroxidase, catalase) activity; 5) polyphenol content; 6) SH content; 7) starch content; 8) sugar content; 9) fat content.

The method of determining pH_n and rH_2 with indicators has been described in detail previously [14, 15]. Redox potential (Eh) was calculated according to the formula $Eh = 30(rH_2 - 2pH)$ in mv [16, 17]. Benzidine, thiourea, and hydrogen peroxide were used in determining activities of oxidative enzymes [15]. Polyphenol content was determined with an alcoholic solution of iron chloride (10% of saturation value); SH content was determined by heating preparations with 3% sodium nitroprusside and a saturated alcoholic solution of zinc acetate. Starch was determined with Lugol's solution (I in KI). Fats were determined by staining sugar preparations with a saturated alcoholic solution of Scharlach red: 1) in conjunction with heating in the presence of Fehling's solution and 2) in conjunction with heating in the presence of 10% α-naphthol and concentrated sulfuric acid.

1. Acidity

The pH_n of freshly collected pollen of Cerasus Vulgaris as determined with neutral red showed considerable variation from grain to grain. Values within the full range of the indicator, 5-7, were observed, and some grains exhibited values beyond this range, especially below 5.

With thymol blue and bromphenol blue as indicators, many pollen grains manifested values of 3 or lower. It was also established that the exine of some of the grains stains a pinkish color with thymol blue, although this occurs very seldom. In general, this staining pattern indicates that the pH of the exine may be as low as 1.2. Some grains stained a yellowish brown with neutral red and a bluish color with brom thymol blue (pH ≈ 7 or higher.

A differential staining of the protoplasm and exine of the pollen was observed, attesting to the well known differences between these parts with respect to pH_n. The exine was usually more acid than the protoplasm (staining with thymol blue and brom phenol blue). It was established, moreover, that there are local differences in pH_n of 1-1.5 units; parts of the pollen grain near the pores were more acid. Such differences were reflected both in color tone and in rate of staining. On the whole, the pH_n of most of the pollen grains of C. vulgaris was between 2.8 and 5. Approximately the same values were obtained for the other species studied, although the distribution of values within the range was naturally somewhat different for different species.

pH_n Indicators

Indicators	Color change	Effective pH range
1. Thymol blue	Red-yellow	1.2-2.8
2. Brom phenol blue	Yellow-blue	3.0-4.6
3. Methyl red	Red-yellow	4.4-6
4. Neutral red	Crimson-yellowish-brown	5-7
5. Brom cresyl purple	Yellow-purple	5.2-6.8
6. Brom thymol blue	Yellow-blue	6.0-7.6
7. Phenol red	Yellow-red	6.8-8.4
8. Cresyl red	Yellow-red	7.2-8.8
9. Thymol blue	Yellow-blue	8.0-9.6

rH_2 Indicators

Indicator	rH_2 of transition from oxidized to reduced form
Neutral red	4
Janus green	5 Transition from red to colorless form
Phenosafranin	6
Cresyl violet	8
Nile blue	9
Janus green	12 Transition from blue to red form
Methylene blue	14
Cresyl blue	15
Thionin (Lauth's violet)	17
Dahlia violet	18

Staining of transverse and longitudinal sections of the stigma, style, and ovary of C. vulgaris revealed that they are finely differentiated with respect to pH_n. The epidermis of peripheral portions of the stigma and a comparatively thin layer of subepidermal cells manifested low pH_n values (from 5 to 3 with neutral red, brom cresyl purple, brom phenol blue). The pH of the central epidermal cells could not be determined with neutral red, since this indicator is reduced and thereby decolorized by these cells. With brom cresyl purple, brom thymol blue, and phenol red, however, it was shown that here the pH is 6.8-7 or higher.

The radial distribution of pH values in the style is the same as in the stigma. Perhaps the sole difference between the style and stigma resides in the fact that the peripheral zone of low pH is considerably larger in the former case. The central tissue of the style, as of the stigma, has a pH from ~ 7 to > 7.

Staining of transverse sections of the ovary revealed that the internal and external epidermis of the ovary and the ovular integuments have a low pH_n (5-5.5, neutral red), while the ovary parenchyma and the nucellus have a higher pH_n (6.8 and higher, brom phenol blue and cresyl red). The same pH_n patterns were found in other members of the Prunoideae.

Of special interest from the morphological point of view were the compound pistils (Malus domestica, Pyrus communis). The pH patterns of the stigma and the free portion of the style were practically the same as those already described for simple pistils. With respect to the fused portion of the style, there were five separate canals of high pH (6-7) through which pollen tubes would grow, surrounded by rings of tissue of low pH_n, <5. As in the previous case, the epidermis was characterized by low pH_n (< 5). It was established in the course of the study that each canal, which passes to the ovary from the free portion of the style, corresponds to a separate carpel. Results of field experiments are in agreement with this conclusion [18].

2. rH_2

By staining pollen of C. vulgaris with various redox indicators, it was established that individual grains vary greatly in rH_2. Values obtained ranged from 8 to 18, although it was not possible to determine the true upper limit since no indicators with rH_2>18 were available to us. On the whole, the pollen of C. vulgaris manifested relatively high values (~ 18 and > 18). Approximately the same values were obtained with the other species examined, although the distribution of values in a pollen population varied from species to species.

The treatment of pistillate tissues of C. vulgaris and other species with redox indicators revealed patterns of no less interest than those obtained with pH_n indicators. In

order to avoid repetition of details touched on in a fair amount of detail in the previous section, we will merely state that those tissues which were characterized by high acidity also yielded high rH_2 values, while those which were more basic gave the lower rH_2 values.

Epidermal and subepidermal cells at the periphery of the stigma and style, epidermal tissues of the ovary, and ovular integuments yielded values of 15 to 18 and higher, while central tissues of the stigma and style, nucellus, and cells of the embryo sac yielded values less than 4 (reduction of neutral red). Pistils with fused styles exhibited rH_2 patterns similar to pH patterns. It should be noted, however, that the decrease in rH_2 from the periphery to the center of the stigma and style was considerably more gradual than the decrease in pH.

Incidentally, the gradual increase in the concentric zone of coloration of transverse sections of stigma and style which occurs as one uses indicators of gradually decreasing rH_2 attests to the fact that we were dealing not with local permeability barriers (since permeability of cells to the stains used is about the same for all of them), but with local differences in oxidative potential.

3. Redox Potential

In view of the fact that we were unable to determine pH_n and rH_2 of a given object simultaneously, we shall present in this section only the extreme values of Eh, which will serve as limiting values indicating the range of variation of this parameter.

These values, which were obtained by calculation, yielded a considerably more clearcut pattern, with greater contrasts, than those patterns which emerged from pH_n and rH_2 determinations. For pollen, the values fell between + 372 and -180 mv, although there were manifestly some cases in which they were beyond these limits. The main body of the pollen, however, had values between +372 and +240 mv. Undoubtedly, there are very fine graduations in Eh of pollen between the calculated limits (Table 1).

The local variations, within a single pollen grain, of pH_n and rH_2 suggest that Eh may vary in a similar fashion. The pistil proved to be even more finely differentiated with respect to Eh.

In Table 1 it is seen that large positive value (+360 mv and higher) are characteristic of the peripheral epidermis of the stigma and the adjacent subepidermal cells, the ovary epidermis, and the ovular integuments; the surface of the stigma, the central tissue of the style, the nucellus, and the cells of the embryo sac are characterized by large negative values (-300 mv or more). The differentiation of pistillate tissues of apple and pear with respect to Eh corresponds to pH and rH_2 values given above.

4. Activity of Polyphenol oxidase, Peroxidase, and Catalase, and Levels of Polyphenols and Sulfhydryl Groups

Differences in oxidative properties of pollen and pistillate tissues were of course manifested in differences in content and activity of such physiologically active substances as oxidative enzymes, polyphenols, which participate in terminal oxidation, and sulfhydryl groups, which are components of cellular redox systems.

In C. vulgaris, these differences were on the whole parallel to differences in pH and rH_2.

To a considerable degree, the activity of the various oxidative enzymes showed characteristic distributions from tissues to tissue. In C. vulgaris pollen, polyphenolase activity was very low (weak blue staining with benzidine), and in some pollen grains it was generally absent. Peroxidase activity was greater, with the pollen grains falling into three groups according to activity level: 1) high activity (intense blue-violet staining); 2) low activity (weak blue staining); 3) no activity (no staining). It should be noted that there are local differences in activity within a pollen grain, which were very clearly indicated by staining intensities.

In the course of the experiments it was found that oxidative enzymes can diffuse through the pollen wall and catalyze substrate oxidation in the surrounding medium. In our view, this is of theoretical significance, since it attests to the fact that during pollination physiologically active materials can easily pass from the pollen to the stigma. Catalase activity, which was measured according to the rate of formation of oxygen bubbles, proved to be quite high, but even here there were differences from grain to grain. Some grains rapidly decomposed hydrogen peroxide, while others were sluggish or completely inactive.

Determinations of polyphenols and sulfhydryl groups indicated that these compounds occur in C. vulgaris pollen in comparatively small amounts. It should be noted that differences in individual grains were fairly well marked.

The pollen of the other species studied was similar to that of C. vulgaris in oxidative enzyme activity and content of polyphenols and sulfhydryl groups. There was a certain degree of variation from species to species but it was not great. Pistillate tissues of C. vulgaris were as heterogeneous with respect to oxidative enzyme activity as to pH, rH_2, and Eh. Those tissues of high acidity, high rH_2 and high positive Eh (epidermis, subepidermal cells) exhibited the highest enzyme activities; those with low acidity, low rH_2, and negative Eh exhibited little or no activity (central tissue of the style, nucellus).

Catalase activity of pistillate tissues was far lower than that of pollen, the distribution from tissue to tissue resembling that of polyphenol oxidase and peroxidase. In the pistil there were even smaller amounts of polyphenols and sulfhydryl groups than in the pollen, these being concentrated mainly in the epidermis and subepidermis of the stigma and style, i.e., in those parts characterized by highly active oxidative enzymes and high pH, rH_2, and +Eh. Oxidative enzyme activity and polyphenol and sulhydryl distribution in the pistillate tissues of the other species were on the whole the same as for C. vulgaris.

TABLE 1. pH_n, rH_2, and Eh of Pollen and Pistillate Tissues

Object	pH_n	rH_2	$Eh = 30(rH_2 + 2pH)$ m
Pollen			
Main pollen mass	2,8--5	≥18	≥+372—240
Minor pollen mass	≥7	~8	≥—180
Pistil			
Epidermis of style, ovary walls	3—5	≥18	≥+360—240
Ovular integuments	<5	~15	≥+150
Stigma epidermis, central style tissue, nucellus	~7	<4	≥—300

5. Starch Content

Staining of the pollen and pistillate tissues of C. vulgaris with an I-KI solution revealed great variations in starch content. Most of the pollen grains contained small amounts, and only a few had relatively large amounts. It was found that the starch is dispersed through the protoplasm, presumably in the form of very small granules, or even in diffuse form. No areas of localized concentration were found. Differences from species to species in pollen starch content were quite considerable; there were large amounts in plum and peach and small amounts in pear and almond. Starch was also present in the pistil of C. vulgaris. It was especially abundant in the central portion of the style through which the pollen tubes would grow, being concentrated mainly in the peripheral area of this cylinder, in the form of elongate inclusions. There was very little starch in the epidermis and subepidermis of the stigma and style, while in the ovary walls and ovular integuments there were considerable amounts. The pattern of starch distribution in pistils of the other species examined was similar to that of C. vulgaris.

6. Sugar Content

The method of choice in determination of sugars was that employing α-naphthol and concentrated sulfuric acid since with Fehling's solution insufficient amounts of cuprous oxide were produced, and the blue-violet color which appeared was an expression of the biuret reaction for protein.

With this method, it was established that there is relatively little sugar in C. vulgaris pollen, and that sugar levels of individual pollen grains varied within a species and among the different species tested. It was difficult to determine the extent of this variation, however, since during heating the pollen grains disintegrated and sugars diffusing into the surrounding medium produced the blue-violet color reaction outside the grains.

Sugar content of C. vulgaris pistils was very high, and the differentiation of tissues in this respect was extremely marked. The largest concentrations occurred in the peripheral cells of the stigma and style, while in the central parts there were no sugars at all. The fall in sugar content from the periphery to the center was very gradual. Here it should be noted that sugars were concentrated essentially in those parts of the style in which there was very little starch, and vice versa. The outer ovary wall was fairly rich in sugar. Passing inwards through the ovary parenchyma toward the ovules, the starch content declines gradually. The ovules contained significant amounts of sugar. The pattern of sugar distribution in pistils of other species was substantially the same as that for C. vulgaris.

7. Fat Content

With the fat indicator Scharlach red, it was found that the fat content of C. vulgaris pollen was in general very low (traces). Some species differences were noted: In apricot, the fat content was relatively higher than in plum and almond. Within a species there was variation in individual grains, but because of the low fat levels these variations were hardly detectable. No fats were found in pistils of C. vulgaris or the other species examined.

Some Characteristics of Pollen Germination on the Stigma and of Pollen Tube Growth in Pistillate Tissues

Since the general pattern of physiological differences between pollen and pistillate tissues was the same for all the species studied, we consider it feasible to describe certain features of pollen germination and pollen tube growth without reference to individual species.

From the material presented it is obvious that in pollination the pollen grains, which have one set of properties (high acidity, high rH_2, high oxidative enzyme activity, large positive Eh), come to lie in contact with the central part of the stigma, which has quite different properties [low acidity (~ 7), low rH_2 (< 4), low oxidative enzyme activity, large negative Eh].

Under natural conditions, the time from completion of pollen maturation in the pollen sacs to pollination is relatively short (but sometimes days in length). During this period metabolic activity of the pollen must be fairly high, and, therefore, reserves of nutrient materials are

requisite for maintenance of this activity. These materials are present in pollen grains in the form of starch, sugars, and, to some extent, fats, which are utilized in respiration.

Upon coming in contact with the stigma surface, the pollen germinates. In our view, this germination process can hardly be regarded as a purely mechanical phenomenon involving the protrusion of intine and protoplasm through a pore in the exine in response to an osmotic gradient between the pollen grain and the external environment. It is, rather, a complex physiological process, the stages of which are delicately coordinated and regulated. Even so, osmotic pressure undoubtedly plays an important role.

The pollen grain contains a complex association of enzymes (dehydrogenases, phenolases, peroxidase, catalase, hydrolase, invertase, etc.). We have established that the pollen membranes are fairly permeable to physiologically active materials, particularly enzymes. It is natural to assume, therefore, that upon pollination a complex of enzymes catalyzing and directing conversions of substrates in the stigma and style passes from the pollen to the stigma by exosmosis. Let us also assume the reverse process, that of passage of physiologically active materials from the stigma surface into the pollen grain by endosmosis. It is fully probable that these materials can in some measure stimulate pollen germination.

In the early stages of germination and pollen tube growth, presumably, the pollen's endogenous reserves are utilized for energy and synthesis. (During prolonged storage, pollen viability is maintained at the expense of these same reserves.) This, among other things, is confirmed by the fact that after pollination there is a rapid conversion of starch to sugar, the most convenient and transportable form of nutrient (mediated, apparently, by amylase of the pollen grain).

It is difficult to assume, however, that pollen tube growth to the embryo sac is supported by the endogenous reserves of pollen alone, since in some cases the style is very long. The redistribution of nutrients in the pistil after pollination indicates that they are in a definite manner utilized in pollen tube growth. The limited nature of pollen reserves is also demonstrated by laboratory experiments. Pollen grains can germinate in pure water, but tube growth is very limited and finally ceases. In a sugar solution, however, the tube may reach a considerable length. From this point of view the pistil should be regarded not as a neutral matrix for tube growth, but as a structurally complex and physiologically active system which provides the pollen tube with growth substrates and orients its growth toward the embryo sac and the egg cell.

The heterotrophic pollen tube soon begins to exhaust its endogenous reserves and grows in the direction of the optimum nutrient supply, i.e., through the style to the egg cell.

Enzymes which diffuse from the pollen tube into the style tissue at the growth front catalyze substrate conversions, such as hydrolysis of starch to sugar, in this area.

The transfer of electrons during pollen tube growth in pistillate tissues proceeds without reversal in the direction from -Eh to +Eh, i.e., from pistillate tissues to the pollen tube. If the growing pollen tube and the pistillate tissue are together regarded as a dynamic coupled redox system, then the normal regime for its functioning can only be maintained under conditions of uninterrupted pollen tube growth in pistillate tissues rich in substrates for oxidative degradation.

It is, of course, impossible to explain pollen tube growth solely in terms of differences between the pollen tube and the pistil with respect to nutrient levels and redox potentials; there are a number of chemotropic and other phenomena which unquestionably play an important role. This is borne out, for example, by the absence of normal pollen tube growth in pistillate tissues and the barrier role of various parts of the pistil in cases of hybridization between distantly related plants. Laboratory experiments on pollen germination in the presence of stigmas and ovaries of closely and distantly related species (+ and - chemotropism) also testify to this.

SUMMARY

Results are presented of an investigation of sexual dimorphism and the progamic stage of fertilization in the principal orchard plants of the Rosaceae, namely cherry, plum, apricot, peach, almond, apple, pear, etc. The characteristics studied were the pH, rH_2, and Eh activities of such oxidative enzymes as catalase, peroxidase, polyphenol oxidase, and content of polyphenols, SH groups, energetic and reserve substances (sugars, starch, fats).

In the various species investigated, the pollen is very diverse with respect to the characteristics enumerated above but as a whole is characterized by a low pH (2.8-5), high rH_2 (> 18), high oxidative enzyme activity, high Eh (+372-240 mv); it contains a certain amount of polyphenols, SH groups, sugars, starch, and in some cases small amounts of fats. The oxidative properties of the epidermis of the lateral part of the stigma, the epidermis of the style, and the subepidermal cells strongly resemble those of the pollen.

Considerable amounts of sugars, but no starch or fats, were found in these parts of the pistil. The surface of the stigma, conducting tissue of the pistil and the embryo sac were characterized by a low acidity (pH = 6.8 and 7), low rH (< 4) and by an almost complete absence of oxidative enzymes, polyphenols, and SH groups. Some starch but no sugars or fats were detected in the conducting tissues.

Pollination is accompanied by the passage of a complex of enzymes to the stigma from the pollen and a redistribution of nutrient substances in the pistil. Furthermore, the pollen tube and conducting tissue change into a coupled oxidation-reduction system and the growing pollen tubes utilize the nutrients of the pistil. Simultaneously a complex physicochemical mechanism be-

comes operative, and orients the growth of the pollen tube toward the ovule.

Many details of this complicated process remain obscure. However, from the foregoing it can be asserted that fertilization consists of a complex chain of physiological-biochemical reactions which require further investigation.

LITERATURE CITED

1. G. V. Kulagina, Trudy Saratovsk. SKhI, 7 (14) [in Russian] (Saratov, 1943).
2. L. G. Arutyunova and G. Ya. Gubanov, Agrobiologiya, No. 6 (1950).
3. E. A. Britikov. Trudy Inst. Fiziol. Rastenii Akad. Nauk SSSR 8, II (1954).
4. P. Jouet – Jatergne Protoplasma-Monographien (1931).
5. I. N. L'vova, Selektsiya i Semenovodstvo, No. 9 (1950).
6. E. G. Minina, Control of Sex in Plants by Factors of the External Environment [in Russian] (Izd. AN SSSR, 1952).
7. G. Kh. Molotkovskii and G. V. Grigotsura, Zhur. Obshch. Biol. 17, 212 (1956).
8. V. I. Ostapenko, Byull. TsGL im. Michurina, No. 4 (1957).
9. S. I. Lebedev, Doklady Akad. Nauk SSSR 59, 5, 987 (1948).
10. P. M. Zhukovskii and Zh. Medvedev, Doklady Akad. Nauk SSSR 64, 1, 141 (1949).
11. Grunt et al., Pchelovodstvo, No. 10 (1948).
12. K. E. Ovcharov, Zhur. Obshch. Biol. 15, 353 (1954).
13. V. I. Ostapenko, Some Aspects of Oxidative Processes in Gametogenesis and Fertilization in Drupaceous Plants. Dissertation [in Russian] (Khar'kov, 1959).
14. V. I. Ostapenko, Izvest. Akad. Nauk SSSR, Ser. Biol., 4, 41 (1955).
15. V. I. Ostapenko, Zhur. Obshch. Biol. 19, 296 (1958).
16. R. Vyurmzer, Biological Oxidation and Reduction [in Russian] (ONTI – NKPT, 1935).
17. Ya. E. Ellengorn, Collected Papers in Memory of Academician Maksimov [in Russian] (Izd. An SSSR, 1950).
18. N. A. Abramov, Developmental Characteristics of Seedlings of Certain Fruit Crops in Relation to Length of Seed Dormancy. Dissertation [in Russian] (Michurinsk, 1952).

CERTAIN PHYSIOLOGICAL PECULARITIES IN GERMINATION OF FRESHLY GATHERED CORN SEEDS

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The physiological ripeness of seeds, i.e., a certain biochemical composition and morphological structure, is an important condition determining seed germination.

The ability of some unripe seeds to germinate has been known for a long time [1,2]. Some workers [3,4,5], while studying the ability of wheat seeds to germinate, noticed that this ability did not develop evenly but skipped. The greatest germination of wheat seeds was observed to be about 30 days after anthesis. Then, at the end of the waxy stage, germination decreased and reached a normal value in the fully ripe stage.

The inability of fresh-gathered, not fully ripe seeds to germinate has been related by investigators to various causes such as physiological, biochemical, and even to morphological features. However, up until now, the low germination rate of freshly gathered seeds has not been fully explained. Investigators in search of explanations have cited exceedingly diverse biochemical process – the status of the anaerobic metabolic system, the degree of water permeability of the cell membrane, the presence of a high percentage of water, insufficient nutrient materials in the endosperm, etc. [2,6,8,9,10].

Study of this problem in corn has been quite inadequate, aside from the general indication that the germination of freshly gathered, unripe seeds is low [2,6,8,11].

After evaluating the incomplete data in the literature on this problem, we set about examining the course of germination of freshly gathered corn seeds during various stages of ripening (from the beginning of the milk stage to full maturity), to relate seed germination to seed water content, and to possibly characterize the status of certain structural materials and physiologically active substances in the embryo and endosperm.

METHODS

The experiments were conducted under field conditions with subsequent laboratory analysis of the seeds. Three corn varieties were used, Khar'kov 23, Voronezh 76 and Hybrid VIR 25, sown at the following times: April 20-23, May 13-15, and May 29-June 2. The different planting times were aimed to provide seeds under uniform conditions of seed formation and ripening. Cob samples were taken during the following eight stages of ripening: 1) initiation of milk stage; 2) milk stage; 3) end of milk stage; 4) beginning of milk-wax stage; 5) milk-wax stage; 6) end of milk-wax stage; 7) wax stage; 8) fully ripe. The corn seeds were germinated by standard methods. The germination value was calculated on day seven.

EXPERIMENTAL RESULTS

The experiments showed differences in the pattern of germination of corn seeds freshly gathered at different stages of ripening. During the two initial phases of seed maturity (initiation of milk and milk stages), seed germination was not high (0-10%). At the end of the milk stage, germination increased to 14-24%. In the beginning of milk-wax stage of maturity seed germination rose sharply (to 38%) in all varieties and sowing dates. Similar results were obtained during all three years of experiment.

In the following stages of seed maturity, a decrease in germination (to 14-29%) was observed – milk-wax, end of milk-wax, wax stages. The germination of freshly gathered corn seeds again rose during the stage of full seed maturity to reach 58% in Voronezh 76.

Experimental data for the three years is shown in Table 1, which demonstrates an interesting fact that we have observed in corn – that there are two germination maxima, one during the beginning of the milk-wax stage of development and the second at full maturity. The figure graphically shows the bimodality of germination in freshly gathered corn seeds. The germination of corn seeds varied between years and times of planting in relation to meteorological conditions, decreasing in wet years and increasing in dry years. The conditions for ripening corn seed influenced the over-all percent seed germination, while the bimodal character of the seed germination behavior remained during all three of the experiment years. No varietal differences in this were observed.

How can this bimodality which we observed in corn seed germination be explained?

TABLE 1. Germination of Freshly Gathered Corn Seeds During Different Stages of Seed Maturity (%) (Average of three years, three varieties, and three sowing dates.)

Stage of seed maturity	Seed moisture	April 20-23			May 13-15			May 29 – June 2		
		V-76	Kh-23	VIR-25	V-76	Kh-23	VIR-25	V-76	Kh-23	VIR-25
Beginning milk	75—77	3	2	1	2	1	0	2	3	1
Milk	69—70	10	7	5	7	6	8	6	10	5
End milk	59—64	16	17	14	14	21	16	24	17	15
Beginning milk-wax	57—60	35	34	31	30	38	32	37	29	28
Milk-wax	45—49	19	21	21	25	23	27	19	17	14
End milk-wax	33—42	19	27	15	20	19	16	17	14	14
Wax	32—33	24	29	17	20	25	20	17	22	12
Mature	30—31	58	37	30	34	35	42	58	45	35

The data in Table 1 show that seed germination fluctuates at the same time that the seed water content uniformly decreases. For example, the water volume in the seeds at the end of the milk stage (59-64%) and at the beginning of the milk-wax stage (56-60%) is very nearly the same while germination increases markedly from one stage to the other. Thus, no definite relationship exists between seed water content and the ability of the seed to germinate. Our data support the contention of Kuleshov [9] and other workers who contend that there is no relation between germination and the amount of water in the seeds.

The works of Bakh, Kursanov, Proskuryakov [12,13, 14], and others, indicate that proteins, starch, and other storage substances in mature seeds accumulate intermittently, which explains the nonuniform uptake of nitrogenous and nonnitrogenous substances, and the special features of conversion which these substances undergo in the seeds. In the process of seed ripening, the effects and vigor of the enzymatic processes vary. It has been shown that the amount of certain enzymes initially rises, reaches a maximum, and again falls.

Therefore, it is of interest to try to study certain physiological indicators which characterize separate stages in the process of corn seed ripening, and possibly to uncover the reasons for the bimodal curve of seed germination. In the first place, we became interested in the reason for the rise in the germination curve during the stage beginning at milk-wax ripeness. In order to clarify this question, we used the histophysiological methods which have been successfully employed by Tsinger [15]. With these methods we attempted to characterize the changes in content of a number of substances which develop in the seeds, such as amino acids, nucleic acids, proteins, sugars, starch, fats, ascorbic acids, sulfhydryl groups, peroxidase, polyphenol oxidase, and cytochrome oxidase. The reactions were tried on slices of freshly gathered corn seed of two varieties, Khar'kov 23 and Voronezh 76.

In this way, certain interesting facts were uncovered which explain, so far as we are concerned, the reason for the bimodality in the germination curve. In almost all reactions in the stage beginning at milk-wax ripeness, the amount or activity of the aforementioned substances distinctly increases. In fact, it is during this very stage that the break in the curve of corn seed germination occurs, as we have found in our experiments.

We will briefly characterize the discrete groups of compounds which we have investigated by means of histochemical techniques.

Proteins. Development of the seeds on the parent plant is accompanied, particularly during the early stages, by a rapid protein synthesis, since proteins are essential not only for structure requirements but are also stored.

Proteins have peptide links which are identified by the biuret reaction. The positive reaction produces an intense violet reaction in the scutellar, radicle, and epicotyl regions of seed sections. Other parts of the seed stain weakly. Seeds of the milk stage stain weakly. The most intensive color occurs in seeds in the beginning of the milk-wax stage of ripeness (Fig. 1a). Seeds in the milk-wax, wax, and fully ripe stages stain more weakly; i.e., during the latter stages of ripening, the proteins are subjected to a conversion which blocks identification of the peptide links.

Sugars. The embryo also includes sugars, although not in large amounts, among nonnitrogenous storage and energy materials. These sugars are localized chiefly in the scutellum and in the form of isolated crystals at the base of the radicle and in the epicotyl bud of the embryo.

Reduced sugars are detected by the Boyarkin reaction. When a large quantity of crystals is present, a reddish-brown color appears; with a small number of crystals, the color is yellow. In seed sections at the milk stage, a light brick-red color has already appeared, and the color reaches a maximum intensity at the milk-wax stage of ripeness (Fig. 1b). In a seed section at the milk-wax stage, a yellow tone, with a weak brick-red tint, is de-

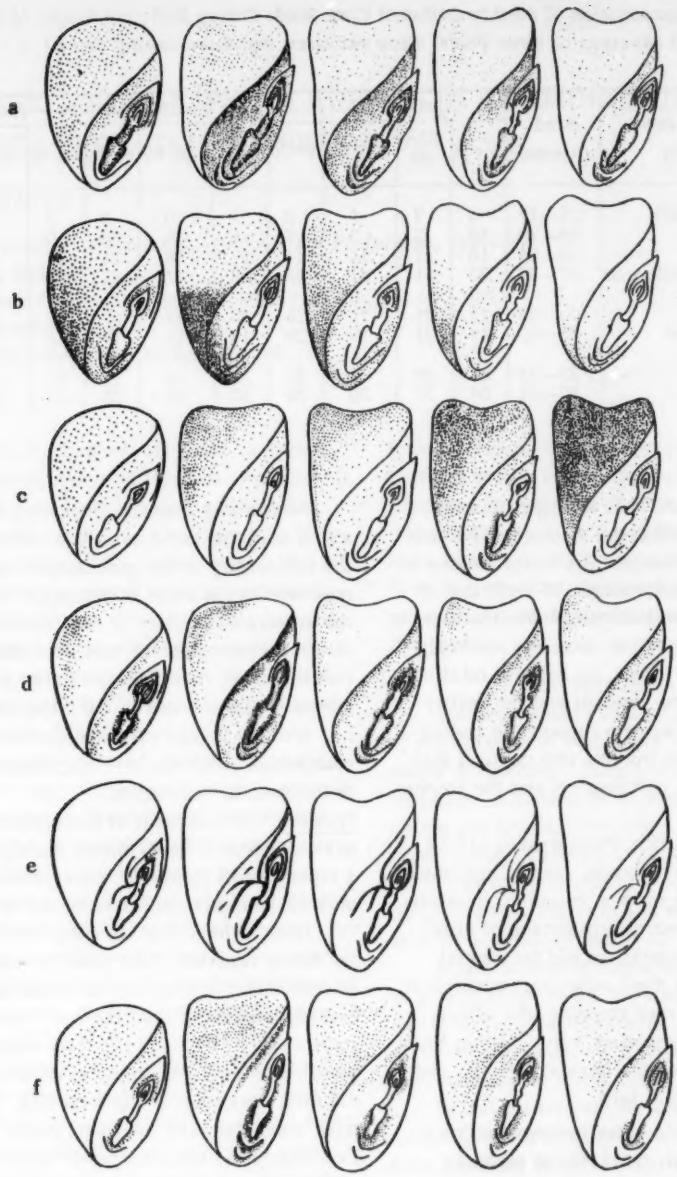

Fig. 1. Reaction to (a) proteins; (b) reduced sugars; (c) starch; (d) ascorbic acid; (e) peroxidase; (f) sulfhydryl groups. Ripeness stages, left to right: milk, beginning milk-wax, milk-wax, wax, mature. Intensity of stippling depicts the degree of staining.

tectable. In seeds of the wax and fully mature stages, the color becomes light yellow, judging by the region of localization, i.e., the amount of reduced sugars decreases as ripening proceeds.

Starch. Nonnitrogenous storage materials are laid down in the form of starch in the endosperm of corn. The central part of the endosperm is not as loaded with storage materials as the peripheral zone (especially in flint corn).

A weak solution of iodine is reacted with starch. The seed sections stain a violet color. The infrequent isolated grains of starch in the endosperm of seeds during the milk stage stain weakly. As the seed matures the color intensity increases and attains a maximum value in fully mature seeds (Fig. 1c).

Sulfhydryl Groups are typified by a high oxidation-reduction lability and universal distribution in tissues.

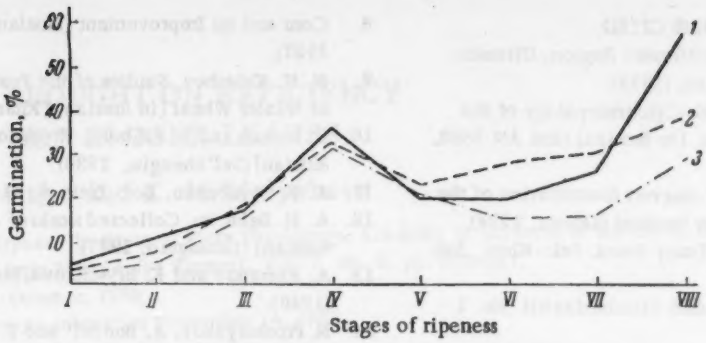

Fig. 2. 1) Hybrid VIR 25; 2) Khar'kov 23; 3) Voronezh 76. Stages of ripeness: I) Beginning of milk; II) milk; III) end milk; IV) beginning milk-wax; V) milk-wax; VI) end milk-wax; VII) wax; VIII) fully ripe.

They are more clearly detected during the early stages of ripening and are localized in the embryo, and somewhat more weakly in the endosperm. In the embryo they are chiefly detected in the scutellum, radicle, and epicotyl bud. The sulfhydryl groups are usually localized where the nucleic acids are found. Lead acetate and sodium nitroprusside in the presence of sulfhydryl groups produce a rose color on the seed sections.

The color is weak in seeds at the milk stage. The most clear reaction appears in the seeds during the beginning of the milk-wax stage. As the seed ages the color intensity decreases (Fig. 1e).

Ascorbic acid possesses very high physiological activity. The amount is large in seed tissues during the early stages of ripening but it disappears as the seed ages and again appears during germination. The reaction to ascorbic acid is based on its reduction by silver nitrate which produces, in the dark, a black or brownish-gray precipitate. Ascorbic acid is localized in the embryo in the scutellum, radicle, and epicotyl bud regions and partly in the endosperm.

In the milk stage, ascorbic acid is present in small amounts in the embryo and along the margin of the endosperm. The most intense color appears in the seed at the beginning of the milk-wax stage. The color weakens as the seed ripens (Fig. 1c).

Peroxidase is a carrier of oxygen to oxidizable substances. The younger the seed, the greater the peroxidase activity. It is detected by its effect on benzidine hydrochloride or o-methoxyphenol in the presence of hydrogen peroxide. In the first case, a blue color appears along the conducting traces in the embryo and in the cylinder of the radicle. In the second case, a brown color appears in the conducting system of the embryo. The reaction also appears in the endosperm.

The color is weak in seeds during the milk stage. The most intense reaction is expressed in seeds at the beginning of the milk-wax stage of ripeness. As the seed ripens, the color weakens (Fig. 1d).

We have briefly characterized the results of histochemical investigations on six groups of substances which have been schematically depicted in the figures presented. Analogous results have also been obtained on other reactions (amine acids, nucleic acids, cytochrome oxidase). With respect to these substances and the enzymes, an analogous parallel has been observed, i.e., the most intensive reactions occur during the beginning milk-wax stage of ripening. Although our investigations cannot pretend to be a complete physiological and biochemical characterization, they cast light upon certain intrinsic phenomena which have a place in the seed ripening process in corn.

SUMMARY

Observation of germination of freshly gathered corn seeds at eight different stages of maturity, beginning from the milk stage up to complete maturity, showed that the germination capacity curve of the seeds has two peaks. The first of these, which attains a value of 38%, refers to the beginning of the milk stage and the second, which reaches 58%, refers to the phase of complete maturity.

Histochemical methods were employed in an attempt to reveal at least some of the physiological-biochemical causes of the first peak in the germination curve.

Histochemical reactions were performed for amino acids, nucleic acids, proteins, fats, reduced sugars, starch, ascorbic acid, sulfhydryl groups, peroxidase, polyphenol oxidase, cytochrome oxidase. The reactions indicated that all substances indicated above (except starch and fats) are most abundant at the beginning of the milk stage of the seeds.

LITERATURE CITED

1. Corn Cultivation in the Ufimskii Region, Ufimskii Leaflet No. 3 [in Russian] (1912).
2. Ya. S. Modilevskii, The Cytoembryology of the Principal Grain Grasses [in Russian] (Izd. AN SSSR, 1958).
3. E. A. Bychikhina, Post-Harvest Germination of the Seed of Grain Crops [in Russian] (Odessa, 1924).
4. V. M. Lebezhninova, Trudy Sverd. Sel.-Khoz. Inst. 3 (1958).
5. L. S. Tret'yakova, Vestnik Standartizatsii No. 1 (1938).
6. V. Crocker and L. Barton, Physiology of Seeds [Russian translation] (IL, 1955).
7. L. I. Sergeev, V. G. Konarev, E. V. Kucherov, and N. S. Kuramshi, in: Problems on the Biology Physiology, and Biochemistry of Corn [in Russian] (Ufa, 1958).
8. Corn and its Improvement [Russian translation] (IL, 1957).
9. N. N. Kuleshov, Studies of the Post-harvest Ripening of Winter Wheat [in Russian] (Khar'kov, 1924).
10. E. Leman and F. Aikhele, Physiology of Seeds [in Russian] (Sel'khozgiz, 1936).
11. A. K. Dzyubenko, Bot. Zhur. 6, 41 (1949).
12. A. N. Bakh, in: Collected works of A. N. Bakh [in Russian] (Leningrad, 1937).
13. A. Kursanov and K. Bryushkova, Biokhimiya 5, 681 (1940).
14. N. Proskuryakov, A. Bundel' and E. Bukharina, Biokhimiya 6, 347 (1941).
15. N. V. Tsinger, The Seed, its Development and Physiological Characteristics, [in Russian] (Izd. AN SSSR, 1958).

EFFECT OF DROUGHT ON EFFICIENCY OF RESPIRATION IN CORN LEAVES

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Biosynthesis, growth, translocation, and absorption of substances is inevitably linked with expenditure of organic substances, and consequently with obtaining energy.

Recently, experiments carried out in determining a whole complex of physiological processes in plants during drought (photosynthetic yield, water regime, growth processes, etc.) have shown a clear discrepancy with an excessively high level of respiration [1,2]. These investigations indicate a decrease in the efficiency of respiration and its yield; i.e., during a considerable water deficit a breakdown of the usual ways of utilizing respiration energy takes place [2-4].

In connection with this, we have carried out a series of field experiments, determining respiration rate and activity of oxidative enzymes on the one hand, and translocation of assimilate on the other, in corn plants undergoing air and soil drought. As controls, plants grown on a favorable water regime were used. The significance of respiration efficiency is more noticeable in the complex process of translocation of nutrient substances than in any other process. The transformation of assimilates into transportable form, the mechanism of translocation itself, the transformation of substances from more transportable forms into less transportable in the places of deposit – all these links of the flow are directly connected with the process of accumulation and transformation of energy, i.e., with respiration efficiency [5].

METHODS AND EXPERIMENTAL CONDITIONS

The experiments were carried out in the most droughty western part of Kulundinskaya steppe (Klyuchevskii region) in the fields of the I. V. Stalin kolkhoz, during the growing seasons of 1955-1956. Two treatments were compared: drought (soil moisture decreased from 40% to 30% during the first half of the growing season, and then dropped to the level of permanent wilting) and irrigation, in which the soil moisture was kept on the average at 70% of total field capacity. Irrigation was carried out through channels along the strips. The irrigation norm was about 800 m^3/ha. The soil in both treatments was the same. Corn plants of varieties Slavgorodskaya 270 (rapid-maturing) and Dnepropetrovsk (average-maturing) were used as experimental objects.

Respiration rate was determined by means of Barcroft differential manometers [6,7]. In each treatment, determinations were carried out in duplicate on sections from the middle part of the leaf of the middle whorl from twenty plants. Exposure was 1 hour; temperature 28°; manometric readings were taken every 15 min. Activity of oxidative enzymes was calculated by the method of Povolotskaya and Sedenko [8], the average sample being composed in the same way as in the case of respiration determination. Determinations were made every five to seven days. The rate of assimilate flow was determined by means of radioactive carbon C^{14} [9]. The car-

TABLE 1. Respiration Rate of Corn Leaves, Variety Dnepropetrovskaya (in mg per hour per g dry weight)

Stage of development	Date of determination	CO_2		O_2	
		non-watered	watered	non-watered	watered
Leaf formation	June 16	37.88	21.88	47.60	25.54
	June 27	22.58	15.92	27.10	24.60
	July 12	19.43	9.78	30.32	12.30
Tasseling	July 17	22.54	18.40	26.50	20.70
Pollen shedding	July 23	16.38	14.28	18.30	16.90
Silking	Aug. 8	17.23	13.28	18.09	15.31
Seed setting	Aug. 14	14.94	13.52	16.42	14.39

TABLE 2. Role of Oxidizing Enzymes in Corn Leaves (in mg of ascorbic acid oxidized per g wet weight in 30 min)

Stage of development	Date of determination	Peroxidase		polyphenol oxidase		Ascorbic acid	
		non-watered	watered	non-watered	watered	non-watered	watered
Variety Slavgorodskaya 270							
Leaf-formation	28.VI	81.8	41.2	61.7	58.7	3.7	1.3
Tasseling	7.VII	592.5	547.5	40.0	27.5	none	none
Anthesis	18.VII	145.0	50.0	131.3	75.0	81.2	75.0
Seed setting	26.VII	448.7	417.5	201.2	118.0	12.5	6.2
The same	3.VIII	310.0	262.5	50.0	37.0	3.1	5.0
Milk stage of maturity	12.VIII	198.9	168.6	35.9	31.4	10.4	9.9
The same	19.VIII	249.0	215.8	33.3	16.6	10.4	8.3
Milk-wax stage of seed maturity	26.VIII	190.5	116.0	108.3	58.3	0	0
Variety Dnepropetrovskaya							
Leaf-formation	23.VI	406	384	29.0	19.0	none	none
The same	28.VI	502	396	15.5	11.5	4.5	1.0
The same	3.VII	380	202	33.5	9.0	8.5	none
The same	8.VII	291	282	9.0	6.4	27.8	15.2
Tasseling	18.VII	919	915	0	0	85.0	9.0
The same	24.VII	825	434	—	—	—	—
Anthesis	4.VIII	256	204	7.2	4.0	2.4	0
The same	9.VIII	259	265	none	none	63.6	62.4
The same	14.VIII	222	110	19.2	12.8	10.8	0
The same	23.VIII	110	263	22.5	19.2	4.5	0
Milk stage of seed maturity	29.VIII	198	160	23.0	18.0	16.0	9.6

TABLE 3. Translocation of Radioactive Carbon from the Leaf into Ears of Corn

Experimental treatment	Stage of plant development	Date of determination	Content of C^{14} in imp/min per mg dry weight					Ratio (1):(2)
			leaf				ear,	
			immed. after feeding (1)	4 hr later	8 hr later	24 hr later (2)	24 hr later	
Nonwatered	Anthesis	16.VIII	110	80	—	40	60	2.75
Watered			4200	1260	—	1080	170	3.88
Nonwatered	Seed setting	21.VIII	830	680	570	490	30	1.69
Watered			1230	800	480	330	90	3.72
Nonwatered	Seed setting	28.VIII	880	500	310	200	—	4.40
Watered			1360	1150	770	160	—	8.50
Nonwatered	Milk stage of seed maturity	5.IX	3150	2180	1100	970	160	3.24
Watered			4030	1400	1000	660	380	6.10
Nonwatered	Milk stage of seed maturity	10.IX	2700	1900	—	1840	40	1.46
Watered			3250	1290	—	580	100	5.51

bon dioxide source was a mixture of regular and radioactive soda, with a specific radioactivity of 1.1 μC/mg The concentration and amount of $Na_2C^{14}O_3$ was selected in such a way that the activity of gas in the chamber was 49.155 μC/liter.

Dimensions of the apparatus had to be somewhat increased to adapt them for the corn leaf. Mixing of soda solution with 50% sulfuric acid (1 ml per determination) was carried out in one arm of the reaction vessel. Samples were collected in duplicate right after "feeding" of the leaf, and after 20 minutes, 1, 4, 8, and 24 hours.

Total radioactivity was determined on a VSP-type 1956-804 torsion counter.

The average sample for determination of soluble sugars [10] was composed of 40 to 50 plants, in whorls.

EXPERIMENTAL DATA

Observations of the respiration rate in corn leaves have shown increase in both O_2 absorption and carbon dioxide evolution during progressive soil and atmospheric drought (Table 1). The activity of oxidases was found to be increased (Table 2).

TABLE 4. Dynamics of Soluble Carbohydrates in the Leaves of Corn, Variety Dnepropetrovskaya, According to the Whorls

Stage of development	Date of determination	Whorl	Total sugars (reducing sugars + sucrose), %	
			non-watered	watered
Leaf formation	Jul.16	1	7.5	7.6
		2	7.4	8.3
		3	11.5	7.7
Anthesis	Jul.31	1	6.5	5.7
		2	7.2	6.2
		3	7.7	4.2
Seed setting	Aug.17	1	8.5	5.2
		2	9.4	6.3
		3	8.0	7.9
	Aug.24	1	7.0	7.7
		2	8.4	7.6
Milk stage of seed maturity	Sept.1	1	5.7	5.7
		2	8.2	6.4
	Sept.7	1	4.4	4.1
		2	4.3	6.4

On the other hand, the experiments show a complete lack of correspondence of oxidizing processes with the rate of translocation of nutrient substances in plants subjected to drought (Table 3).

The rate of assimilate flow from the leaf [Table 3, ratio (1): (2)], as well as general rate of translocation, during strong water deficit is one-third to one-half that than during optimal supply of water.

The decrease in assimilate in plants undergoing drought (measured by hours) is considerably slower, in spite of the increased expenditure of substances for respiration (Table 1). The great accumulation of soluble carbohydrates in leaves, according to the whorls, (Table 4), together with their simultaneous considerable expenditure is an indirect confirmation of the poor translocation of nutrient substances along the plant during drought. It is also interesting to note the decrease in respiratory quotient (Table 5) in plants undergoing drought during vegetation.

DISCUSSION OF RESULTS

Increasing soil and atmospheric drought during the process of vegetation causes breakdowns in the energy metabolism of plants. A sharp decrease in carbon assimilation [Table 3, column entitled "immediately after feeding" - (1)], with simultaneous increased oxygen absorption and increased activity of oxidizing enzymes, is accompanied by a very weak transportation of photosynthetic products. Consequently, during drought plant respiration loses its effectiveness. The level of oxidizing processes does not correspond to the synthetic ones, while processes directly connected with the expenditure of energy (outflow of the assimilation products) are in an inhibited state. The decrease of the respiratory quotient

TABLE 5. Respiratory Quotient in Corn Leaves, Variety Dnepropetrovskaya Under Conditions of Drought and Optimal Water Regime

State of development and determination	Date of determination	Respiratory quotient	
		non-watered	watered
Leaf formation	July 16	0.79	0.86
	Aug. 7	0.73	1.00
	Aug. 12	0.64	0.78
Tasseling	Sept. 3	0.79	0.90
Silking	Sept. 14	0.89	0.91
Milk stage of seed maturity	Sept. 23	0.79	0.87
	Sept. 29	0.69	0.76

during drought is a curious fact, which indicates either a change in the nature of the respiratory material, or an incomplete oxidation. We are more inclined to favor the second possibility, because of the increased accumulation of soluble carbohydrates in the leaves of the unwatered treatment (Table 4).

The weak rate of outflow of assimilation products from the leaf during deep water deficit, the decrease in the general rate of translocation to half or one-third of that of watered plants, the small decrease in assimilates in the leaves in the course of hours, together with the simultaneous increased rate of respiration and oxidizing enzyme activity indicate a decrease in respiration efficiency, a breakdown in the complex system of oxidation-reduction reactions, the nonconstructive usage of energy material, i.e., a physiological devaluation of respiration.

SUMMARY

1. Progressive soil and atmospheric drought causes a superfluously high increase in respiration rate and oxidative enzyme activity (peroxidase, polyphenol oxidase, ascorbic acid oxidase), as compared with the level of synthetic processes.

2. The increase of the oxidative processes is accompanied by a simultaneous sharp decrease of assimilate flow out of the leaf, both in rate of flow as well as in the amount of assimilants.

3. During moisture deficiency in plants, one notes a decrease of the respiratory quotient, evidently because of the incomplete oxidation of energy material.

LITERATURE CITED

1. N. S. Petinov, Izvest. Akad. Nauk SSSR, Ser. Biol. No. 5, 1 (1954).
2. V. N. Zholkevich, Fiziol. Rastenii 2, 123 (1955).
3. V. N. Zholkevich, Coll.: Biological Bases of Irrigated Agriculture [in Russian] (Izd. AN SSSR, 1957).
4. V. N. Zholkevich, Doklady Akad. Nauk SSSR 121, 6, 1093 (1958).*
5. V. N. Zholkevich, L. D. Prusakova, and A. A. Lizandr, Fiziol. Rastenii 5, 337 (1958).*

*See English translation.

6. D. A. Sabinin, Physiological Bases of Plant Nutrition [in Russian] (Izd. AN SSSR, 1955).
7. I. V. Kozhanchikov, Experimental-Ecological Methods of Investigation in Entomology [in Russian] (VASKhNIL, Leningrad, 1937).
8. W. W. Umbreit, R. K. Burris, and J. F. Stauffer, Manometric Methods of Tissue Metabolism Study, Ed. V. A. Engel'gardt [Russian translation] (Moscow-Leningrad, 1951).

TRANSLOCATION OF CALCIUM IN PLANTS

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Mineral translocation in plants was first studied by Mason, Maskell, and Phillis [2-5]. In these investigations, a downward translocation of calcium through the stem cortex could not be demonstrated.

We have performed a series of experiments using Ca^{45} in which the isotope was introduced directly into the cortex and then traced by radioactivity measurements as it moved into other parts of the plant.

A ring of bark was removed from the stem and replaced by a plastic strip with a U-shaped cross section, the exposed part of the cortex having first been covered with cotton soaked in a solution of calcium chloride containing Ca^{45}.

In one experiment, calcium was introduced in this manner into the stem cortex of blooming cotton plants and after five days the radioactivity of various parts above and below the site of application was measured. Results of these determinations are presented in Table 1.

The data clearly indicate that calcium introduced into the stem cortex is translocated only in an upward direction, with the result that it accumulates to a marked extent in the upper parts of the plant. The greatest accumulations are in the young tissues. For example, total radioactivity of the leaf at the fourth node of the main stem was 571 imp/min·g wet weight, while the young leaf adjacent on the axillary shoot had an activity of 109,235 imp/min.

Experiments with cotton were repeated several times, translocation being studied in the stages of floral initiation, fruit formation, and fruit maturation. At no time was movement in a downward direction observed.

Experiments on calcium translocation in lilac shoots during the blooming period and the period of vegetative growth were also carried out. In one such experiment, the radioactive isotope was introduced into the cortex on April 21, and samples for determination of radioactivity were collected on April 27.

The radioactivity of portions of the plant above the site of application was found to be as follows:

Plant part	Radioactivity, imp/min·g wet wt
Cortex	29000
Leaf	10585
Flower buds	1400

No radioactivity was detected below the site of application, and thus, as with cotton, calcium was translocated through the stem in an upward direction only. Similar results were obtained in experiments with other plants as well.

Special experiments with two-year-old shoots of Chinese rose were carried out in order to clarify the role of the cortex in calcium translocation along the stem.

The wood was removed from the base of 10-cm shoots and these shoots were immersed in a solution of radioactive calcium so that the lower 3 cm of cortex was covered. In order to prevent leaf desiccation, the

TABLE 1. Radioactivity of Various Plant Parts after Introduction of Ca^{45} into the Cortex

Position of plant parts	Plant parts	Activity, imp/min · g wet wt
Above the application site	Old leaf, fourth node of the main stem	571
	Young leaf on shoot from the fourth node	109235
	Cortex	135380
	Wood	19720
Below the application site	Leaf on the main stem	0.0
	Cortex	140
	Cortex, stem base	0.0

TABLE 2. Radioactivity of the Cortex and Wood of Chinese Rose Shoots after Exposure to a Solution of Radioactive Calcium

Nodes, from below	Radioactivity, imp/min · g wet wt	
	Cortex	Wood
Parts in solution	418500	—
2-3rd internode	2205	0.0
4-5th internode	7500	0.0
6-7th internode	—	0.0
7-8th internode	8031	291
Apex	4354	200

shoots were kept for four days under a glass bell jar lined with wet filter paper. On the fourth day samples of cortex and wood were taken from various parts of the shoot for determination of radioactivity. Results of these determinations are presented in Table 2.

Regardless of the absence of wood at the stem base, calcium is translocated upwards and accumulated in the upper regions of the shoot in large amounts. This, together with the absence of radioactivity in the wood of lower stem parts, provides incontrovertible proof that translocation occurs in the cortex alone. A small amount of radioactivity is found in the wood of the upper regions of the shoot, and this evidently results from translocation from the cortex to the wood.

To clarify the role of wood in upward translocation, one experiment with lilac was performed in which the radioactive material was applied to the cortex in the middle of the stem, after which the stem was girdled above this point. Five days later, samples were taken from various parts of the stem for measurement of radioactivity. Results were as follows:

Plant parts	Radioactivity, imp/min · g wet wt
Cortex above site of application and below girdle	57866
Cortex above site of application and above girdle	10923
Leaf above site of application and below girdle	8324
Leaf above site of application and above girdle	27694
Cortex below site of application	0.0

These results show that under these conditions calcium was translocated only upwards and that girdling of the stem above the site of calcium application did not hinder this process. Those parts above the girdle showed a marked increase in radioactivity; in this case translocation was through the wood instead of the cortex, indicating that calcium introduced into the cortex can easily pass into the wood and move through the dead tracheids and vessels. It should be pointed out, however, that although girdling the stem did not prevent upward translocation, it strongly impeded it. As a result we observe in girdled plants huge accumulations of calcium in the cortex below the girdle. The presence of radioactivity in the cortex above the girdle shows that the calcium which had been translocated past the girdle through the wood then passes into the cortex and accumulates there in fairly large amounts. Thus, calcium is translocated upwards both through the cortex and the wood, with lateral movement in both directions taking place.

Having established the upward translocation of calcium through the stem cortex, we passed to the elucidation of the underlying reason for the fact that calcium, in contrast to other elements, is translocated upwards only. We attempted to explain this in terms of the character of distribution of calcium upon entering the plant. As we have already seen in Table 1, it is accumulated mainly in the young tissues, being translocated to the old tissues in small amounts. This pattern of distribution is characteristic of the plant as a whole and also of its individual organs. We shall present results of experiments with narcissus designed to reveal distribution patterns in leaves. This plant was dug up in the spring and its root system immersed in a solution of Ca^{45} containing calcium chloride for three days. On the fourth day samples were taken from various parts of the plant for determination of radioactivity. Results are presented in Table 3.

Table 3 shows that calcium entering the leaf is accumulated exclusively in the young parts, being completely absent from the upper, older parts.

In view of the rapid absorption of calcium by young tissues, we thought it possible to alter the direction of flow of this element by removal of young growing parts and suppression of apical growth. In one experiment with cotton, the apices and axillary shoots in the upper part of the stem were removed. After some time, the lower parts began to grow, and after the appearance of young shoots calcium was introduced into the cortex at the

TABLE 3. Distribution of Calcium in Various Parts of the Narcissus Leaf

Leaf parts	Radioactivity, imp/min · g wet wt	
	Second leaf	Third leaf
Youngest lower part	41875	34023
	41985	25027
Young lower part	17076	3175
Middle part	3296	211
Old upper part	900	207
Oldest upper part	0.0	0.0
Oldest upper part	0.0	0.0

middle of the stem. Four days later samples were taken for determination of radioactivity. Results were as follows:

Plant parts	Radioactivity, imp/min · g wet wt
Cortex above site of application	136925
Leaf above site of application	10478
Cortex below site of application	0.0
First leaf below site of application	0.0
Fourth leaf below site of application	0.0

These data show that upward translocation of calcium through the stem is determined by the physiological status of the stem itself and of its parts. We assumed that the phenomenon is related to the existence of an acropetal concentration gradient of mobile forms of various calcium compounds in the stem cortex. At first glance this assumption seems to contradict our previous conclusions of high levels of calcium compounds in the upper parts of plants [1]. In this study, however, it was established that the major part of the calcium in these regions occurs in bound form. Absorption of calcium by these young cells is therefore possible, and this calcium binding promotes the development of a concentration gradient along the stem, increasing from the base to the apex. In view of this, we thought it possible to alter the direction of translocation by altering the direction of the concentration gradient, this being accomplished by sprinkling the upper plant parts with a calcium chloride solution. To this end an experiment was performed in which the upper part of a cotton plant was sprinkled with a 2% solution of calcium chloride, and after a few hours radioactive calcium was introduced into the cortex at the middle of the stem.

Analysis of the distribution of radioactivity in this plant yielded the following results:

Plant parts	Radioactivity, imp/min · g wet wt
Cortex above site of application and below the node	861871
Wood above the site of application and below the node	33225
Cortex above the site of application and above the node	247636
Wood above the site of application and above the node	12179
Old leaf of the sixth node above the site of application	461
Young leaf of the sixth node above the site of application	28400
Cortex below the site of application and above the node	58592
Cortex below the site of application and below the node	1176
Wood	2923
Leaf of the fourth node below the site of application	5250
Leaf of the fifth node below the site of application	4768
Cortex at the stem base	272
Wood at the stem base	0.0

These results show that with calcium enrichment of the upper parts of the plant in the manner described, there is induced a downward translocation of calcium subsequently introduced into the cortex; radioactivity of organs below the site of application is perceptibly increased.

The downward translocation is of limited extent and no calcium is found at the stem base. Evidently there is a rapid lateral movement from cortex to wood, after which the element is again translocated upwards. These data clearly show that direction of translocation along the cortex is determined by the direction of the calcium

concentration gradient. In the stem of unsprinkled plants, where there is an acropetal concentration gradient of mobile calcium compounds, radioactive calcium is translocated upwards. A reversal of the direction of the gradient resulted in the addition of a downward translocation component.

SUMMARY

1. Calcium is translocated through the stem cortex mainly in an ascending direction. During translocation, it can pass from the cortex into the wood and move through it as well.

2. Upward translocation of calcium through the cortex is manifested by the acropetal gradient of calcium compounds, this gradient depending on the binding of calcium by protoplasm of young tissues. Saturation of the upper parts of the plants with calcium by spraying with a calcium chloride solution promotes downward translocation as well.

LITERATURE CITED

1. M. G. Abutalybov. Fiziol. Rastenii 3, 306 (1956).
2. T. Mason and E. Maskell, Ann. Bot. 45, 126 (1931).
3. T. Mason, E. Maskell, and E. Ptillis, Ann. Bot. 50, 23 (1936).
4. E. Phillis and T. Mason, Ann. Bot. 50, 161 (1936).
5. E. Phillis and T. Mason, Ann. Bot. 59, 679 (1936).

FOLIAR FEEDING OF CORN WITH NITROGEN AND PHOSPHORUS AT VARIOUS LEVELS OF ROOT NUTRITION

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Foliar feedings of agricultural crops, particularly with nitrogen, do not always give positive results. While one of us was studying the characteristics of nitrogen feeding of plants through the leaves [1,2], he showed that spraying corn plants with a 4 to 5% solution of urea causes retardation of nitrogen absorption by the roots for one or two weeks, and sometimes even more. Such retardation can be explained by the fact that when nitrogen is introduced into the leaves, the photosynthetic products are used by the leaves themselves for acceptance of amino acid groups, and the sugar content of leaves decreases. This weakens the flow of assimilants to roots and makes them less able to assimilate soil ammonium. In these experiments, the absorption of nitrogen by the roots was studied for a relatively short period of time – for not more than 14 days after spraying.

The purpose of the present study was to examine the effect of foliar nitrogenous supplementary feeding on absorption of nitrogen by the roots over the course of a longer time period, at various levels of nitrogen nutrition through the roots.

METHODS

The experiments were carried out in 1959 in the greenhouse of the Institute of Plant Physiology, Academy of Sciences of the USSR. Plants of early-maturing corn, variety Spasovskaya, were grown in metal containers holding 5 kg of absolutely dry soil. The soil moisture, about 70% of full field capacity, was maintained by means of daily watering on a weight basis.

The plan of the first experiment was the following: 1) control, spraying with water; 2) double spraying with 3% urea solution; 3) the same as in the second treatment plus 3% NaH_2PO_4. In all cases, the wetter OP-7 was added in concentrations of 0.2%.

The experiment was carried out at high and low levels of root nutrition. High level was made by adding NH_4NO_3 and KH_2PO_4 to the soil (300 mg N and 200 mg P_2O_5 per kg absolutely dry soil), in one case for 3 weeks and in another case for 2 weeks prior to the experiment with foliar feeding. At the lower level of root nutrition no fertilizers were added to the soil.

At the stage of the beginning of tassel emergence, the experiment with foliar feeding was initiated: the first was made on July 13, and the second on July 17. Five days after the second spraying, the first sample of plants was taken: leaf – the fifth from the top, and stem – the internode of the fifth leaf. Subsequent samples were taken every 7 to 8 days. Every sample consisted of 10 plants (Table 1).

Altogether, five samples were taken, i.e., nitrogen introduction through the roots was measured for 38 days after spraying. Samples, from both the sprayed and control plants, were washed with water, then fixed with hot steam, dried at 60° and analyzed. Together with this, the sap flow was collected for 24 hours from decapitated

TABLE 1. Time of Sampling for Analyses

Sample No.	Dates*	Stage of development
1	Aug. 21-22	Tassel emergence
2	Aug. 29-30	Silking
3	Sept. 5-6	Seed set
4	Sept. 12-13	The end of seed set – beginning of milk stage of maturity
5	Sept. 20-21	Milk-wax maturity

* The first number is the date of sampling at the high nutritional level, the second – at the low nutritional level.

plants. From the volume of the sap and the nitrogen concentration (Kjeldahl + nitrate) in it, the amount of nitrogen absorbed by the plant through the roots was calculated.

RESULTS

The effect of foliar feedings on plant yield is shown in Table 2.

At the high level, the increase of yield in aerial mass after aerial leaf feeding was insignificant. The yield increase at low level was somewhat greater; also, while the addition of phosphorus to urea during spraying had no effect on the yield at all at the high level, at the low level the phosphorus had a definite quality effect: with nitrogen, the wet weight of plants increased by 10%, and with N+P the wet weight increased by 18%. The introduction of nitrogen from the roots in sprayed plants, expressed in percent of controls, is shown in Fig. 1.

According to the data on nitrogen absorption, the first thing one can conclude is that, in all four cases, in the first 1 or 2 weeks after spraying the absorption of nitrogen by roots was noticeably decreased in plants receiving supplementary feeding, which confirms the results of our previous investigations [1,2]. In Fig. 1, it can also be seen that after the initial decrease in nitrogen absorption in sprayed plants, an increase in absorption takes place, which continues for 1 to 2 weeks; after this, a decrease in absorption again occurs. The addition of phosphorus to urea during spraying (in equal ratio of N and P_2O_5, the active principle) did not change the general nature of the nitrogen absorption curve (Fig. 1, C and D), although it had some effect on nitrogen absorption, which was expressed in the following: 1) retardation in nitrogen absorption took place one week later than in the treatments with nitrogen alone; 2) at the high level, the addition of phosphorus (Fig. 1, C) somewhat decreased nitrogen absorption by roots at all times and the curve fell somewhat lower; on the low level (Fig. 1, D), on the contrary, the addition of phosphorus increased nitrogen absorption in the second and third week after spraying. From this, one may conclude that the yield increase of aerial mass when phosphorus is added to nitrogen is greater on low level than on the high level (see Table 2) because of these effects.

Alteration of retardation and increase of nitrogen absorption by roots is evidently connected with an unequal flow of assimilants from leaves, which may be judged by the change in sugar content of stems and leaves (Table 3). The change in sugar content of the leaves of the nitrogen treatment (monosaccharides, sucrose, and some of the water-soluble sugars), expressed in percent of control, is shown in Fig. 2. In order to compare changes in sugar content with change in nitrogen absorption, the curves of nitrogen absorption by roots are plotted on the same coordinates.

As can be seen from the data given, the total water-soluble sugars change insignificantly after foliar nitrogen feeding. Sharper changes occur in the content of sucrose and monosaccharides, and here one can see a rather sharp direct connection between the change in sucrose content of stems and nitrogen absorption by roots: as the sucrose content decreases so does the nitrogen absorption by roots. The monosaccharide content, however, increased, which may indicate that in photosynthesis of amino acids, only a part of the sucrose molecule was used, and unused hexoses were accumulated in the stem.

The increased use of sucrose as compared to monosaccharides can be explained by the fact that sucrose, as the most active form of sugar, has a high bond energy [3]. It has been established that sucrose is considerably richer in free energy than maltose and monosaccharides. In the sucrose molecule the two hexose halves are very active; the energy of their bond is 6,500 calories per mole.

TABLE 2. Effect of Foliar Feeding on Yield of Corn

Treatment number	Aerial feeding treatment	Average wet weight per plant, g	The same, % of control
	High level		
I	Control (sprayed with water)	190	100
II	Nitrogen	203	107
III	Nitrogen-phosphorus	198	104
	Low level		
IV	Control (sprayed with water)	104	100
V	Nitrogen	115	110
VI	Nitrogen-phosphorus	123	118

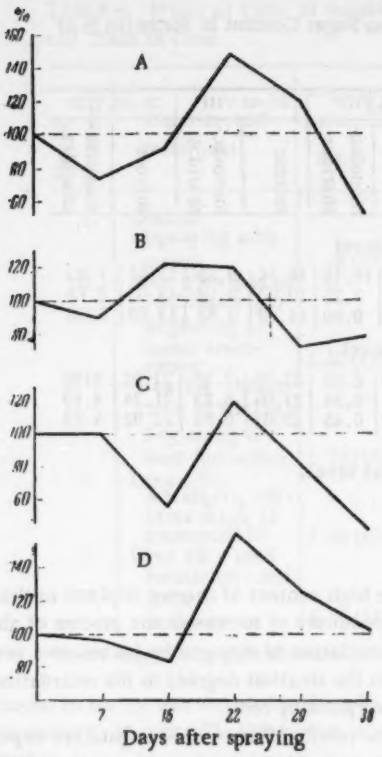

Fig. 1. Effect of foliar supplementary feedings with urea on nitrogen introduction into roots. (Controls were taken for 100%). A and B) Sprayed with nitrogen; C and D) sprayed with nitrogen and phosphorus; A and C) high nutritional level; B and D) low nutritional level.

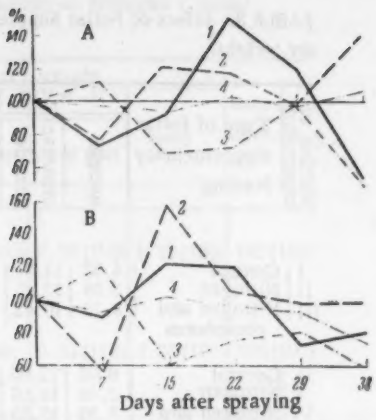

Fig. 2. Change of sugar content in stems and nitrogen introduction into roots as related to the foliar supplementary feedings with urea. (Control plants are taken as 100%). A) High nutritional level; B) Low nutritional level; 1) Introduction of nitrogen from the roots; 2) sucrose content; 3) monossaccharide content; 4) total water-soluble sugars.

The relationship between sucrose content of stems and nitrogen absorption by roots during foliar nitrogen feedings evidently can be explained by the fact that sucrose is the main transport form, and the decrease in its content in stem indicates a decrease in the flow of assimilants to the roots.

The fact that it is particularly the sucrose content that decreases during foliar nitrogen feeding has been noted by Kalinkevich [4], who worked with corn. Eaton and Ergle [5] have shown that when cotton is sprayed with urea, the sucrose content of leaves sharply decreases, while the content of glucose and starch increases.

However, entirely opposite indications can be found in the literature. Thus Borodulina and Runov [6] note that in melons given foliar feeding of a 1.5% solution of ammonium nitrate, the content of monosaccharides was decreased while the content of sucrose and total sugars was increased. An increase in sugar content during foliar nitrogen feeding has been noted by Serbin [7] in the leaves of sugar beet.

Such contradictory data on the effect of foliar nitrogen feeding on the sugar content of the plant probably should be explained by the fact that the samples were not taken in the same periods of time after spraying. Indeed, if in our experiment (Fig. 2), the sample was taken only a week after spraying, it would have been possible to say that the supplementary feeding decreased the content of sucrose; however, if the sample were taken 2 weeks after spraying, then it would have been possible to draw the opposite conclusion – that the sucrose content is increased after the feedings.

The increase of sucrose content in the plants sprayed with urea observed in our experiment 2 weeks after spraying (following the decrease of its content) takes place evidently for the following reason. The spraying of plants with nitrogen, as is known, causes an increase in photosynthetic rate [8-9]. While in the first week, in spite of this, the content of sucrose decreased because of the intensive expenditure of this substance in the process of absorption and nitrogen assimilation, in the second week the increase of the photosynthetic rate exceeded the expenditure of sucrose, which led to the increase of its content.

As far as treatments of spraying N+P are concerned, the addition of phosphorus somewhat destroyed such a direct relationship between the change in sucrose content and absorption of nitrogen by roots.

TABLE 3. Effect of Foliar Supplementary Feedings on Sugar Content in Stems (in % of dry weight)

Number of treatment	Type of foliar supplementary feeding	21—22.VII*		29—30.VII*		5—6.VIII*		12—13.VIII*		20—21.VIII*	
		sucrose	monosaccharides	sucrose	monosaccharides	sucrose	monosaccharides	sucrose	monosaccharides	sucrose	monosaccharides
	High nutritional level										
I	Control	4.92	11.07	10.84	14.25	18.71	10.16	10.36	9.58	13.51	7.25
II	Nitrogen	3.66	12.15	12.99	9.74	21.86	7.25	10.03	9.16	18.65	3.73
III	Nitrogen and phosphorus	3.73	10.62	14.84	12.37	18.76	9.50	11.09	9.33	13.69	6.58
	Low nutritional level										
IV	Control	6.02	12.65	10.44	15.23	23.58	8.55	27.35	7.16	21.22	6.16
V	Nitrogen	2.46	15.07	16.39	9.12	24.93	6.54	27.04	5.73	21.24	4.19
VI	Nitrogen and phosphorus	3.38	15.55	10.85	13.80	25.11	6.45	23.07	6.84	22.92	4.73

* First figure—date of sampling at the high nutritional level.

The relationship noted in our experiment between sucrose content in the plant during foliar feeding and nitrogen absorption by roots may be used for predicting the efficiency of foliar nitrogen feedings. They will probably be more effective when the plant has the greatest content of sucrose. It may be supposed that the foliar nitrogen feedings may be more effective in plants in which the greatest part of sugars is in the form of sucrose. Thus, the hypothesis proposed earlier [1] may be made more precise; It may be said that one of the basis internal factors determing the effectiveness of foliar nitrogen feeding is the content of sucrose in the plant at the moment of feedings, and the existence of conditions for its synthesis in the plant.

The sucrose content in plants changes rather sharply. Thus, in our experiment the sucrose content in the stem during emergence of the tassel was 5 to 6%, while in the phase of grain formation – the beginning of milk maturity it was increased by 3 or 4 times (up to 18% at the high level and up to 24-27% at the low level). Then, in the phase of milk wax maturity, the sucrose content was again decreased (see Table 3).

Our data on the change of sugar content in corn in ontogenesis agree with the data of other authors. Thus, in the field experiments with corn, Krasnodarskii hydrid I/49, which were carried out in the Moscow vicinity [10], the sucrose content in the stems during the phase of tassel emergence was 5.52%; then its content increased in the flowering phase up to 13.44%; at milk maturity, up to 18.49%, after which the decrease in sucrose content began to occur.

Thus, the greatest content of sucrose in corn was observed during the time from silking to milk maturity. At precisely these stages, as one of us noted earlier [1,2], foliar nitrogen feedings give the best effect insofar as accumulation of nitrogen in the plant is concerned. During the high content of sucrose in plants in this period, the expenditure of sucrose in the process of absorption in the assimilation of nitrogen by leaves does not lead (or leads in the smallest degree) to the retardation of nitrogen absorption by roots.

The results of the second vegetative experiment, carried out in 1959, also have shown the advantage of late foliar feedings. The experiment was carried out at the same fertilizer levels, with the same soil as in the first experiment. In the ten-leaf stage, NH_4NO_3 and KH_2PO_4 were applied to the soil of all containers at the rate of one norm of N and P (150 mg N and 100 mg P_2O_5 per kg of absolutely dry soil), and during the phase of tassel emergence, an additional 0.5 norm of N and P.

In our experiment, the foliar feedings were given in 3 periods (for the plan of the experiment see Table 4). At every period, 3 sprayingswere made with 4% urea solution. During the middle period, the treatment included spraying with 4% urea solution plus 4% NaH_2PO_4 solution. There were 16 plants in every treatment, one plant per container. The experiment was concluded at the phase of wax maturity. For analysis, we took leaves (fourth from the top and the leaf next to the ear shoot), stem (internodes of these leaves) and grain. Leaf and stem samples were washed with water.

The experimental results are given in Table 4.

It can be seen from Table 4 that the later foliar feedings are given, the more effective they are, insofar as increase in nitrogen content of the plant is concerned. The sharp difference in the content of nitrogen between upper and lower leaves is very noticeable and can be explained by the fact that the lower leaves are older and they begin to yellow. Nevertheless, in the lower leaf one notes the same relationship of change of nitrogen content in relation to the time of feeding as in the upper leaves. During late feedings the increase in content

TABLE 4. Effect of Time of Supplementary Foliar Feedings on Nitrogen Content and Yield in Corn

No. of treatment	Experimental treatment	Total nitrogen, % dry weight					Wet wt. of plant, g
		leaves		stems		seed	
		upper	lower	upper part	lower part		
I	Control (spraying with water)	1.28 (100)	0.83 (100)	0.43 (100)	0.30 (100)	1.22 (100)	182 (100)
II	Urea 4% - end of leaf formation - beginning of tassel emergence	1.55 (121)	0.90 (108)	0.42 (98)	0.34 (113)	1.24 (102)	186 (102)
III	Urea 4% - beginning of silking - beginning of seed formation	1.73 (135)	1.29 (155)	0.40 (93)	0.38 (127)	1.40 (115)	196 (108)
IV	Urea 4% + + NaH_2PO_4 4% - same stage as treatment III	1.59 (124)	1.15 (138)	0.30 (91)	0.35 (117)	1.46 (120)	179 (98)
V	Urea 4% - seed formation - milk stage maturity	1.90 (148)	1.34 (161)	0.52 (121)	0.44 (147)	1.53 (125)	185 (102)

Note: Figures in parentheses are in percent of control.

of total nitrogen in leaves was almost 1½ times higher as compared to controls, in stems 21-47% higher, and in grain 25% higher.

The field experiment, carried out in 1959 in Krasnodarskii region with foliar feedings in the same stage of development as in the vegetative experiment, in spite of the very droughty summer, showed an analogous relationship in accumulation of protein in plants: the late feedings were more effective than the early ones. But the degree of increase in nitrogen content in corn was not so high; in leaves the nitrogen content increased by 14-18%, in the stems by 12-30%, in the grain by 8% (A. N. Pavlov, E. G. Greenfeld).

To return to the data of Table 4, it should be noted that only in the period of silking – formation of grain in treatment III the foliar feedings have somewhat increased the yield of the total mass of plants (by 8%). Insofar as treatment IV is concerned, where nitrogen-phosphorus foliar feedings were given, it can be said that the addition of phosphorus to urea did not have any positive effect either on nitrogen accumulation or yield; on the contrary, a certain decrease of yield has been noted as compared to the feeding with urea alone. These data agree with results of the first experiment, where it was shown that the addition of phosphorus to urea during spraying of plants grown at the high level did not decrease nitrogen absorption by roots (Fig. 1, C).

SUMMARY

1. In corn plants sprayed with 3% urea solution in the phase of the beginning of tassel emergence, the absorption of nitrogen by roots was studied (by the method of sap analysis) for 38 days after spraying.

2. Foliar nitrogen feedings caused a decrease in nitrogen absorption by roots for 1 to 2 weeks, which then changed to an increase in absorption, which also continues for 1 or 2 weeks, after which a decrease in nitrogen absorption by roots again took place.

3. A direct relationship was noted between content of sucrose in stems and leaves of sprayed plants and nitrogen absorption by roots.

4. Retardation of nitrogen absorption by roots during foliar feedings is connected with a decrease in the flow of assimilants, mainly sucrose, to the roots.

5. Foliar feedings with urea give their greatest effect, insofar as maximum accumulation in the plant is concerned, during the period from silking to milk maturity of grain, when leaves and stems contain their greatest concentration of sucrose.

6. Foliar feedings were more effective at the low level of root nutrition, rather than at the high level. The addition of phosphorus to urea somewhat increased the effect of foliar feedings at the low level.

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LITERATURE CITED

1. A. N. Pavlov, Fiziol. Rastenii 7, 3, 326 (1960).*
2. A. N. Pavlov, Doklady Akad. Nauk SSSR 134, No. 2 (1960).*
3. A. L. Kursanov, Uspekhi Biol. Khim. 2, 220 (Medgiz, 1954).

*See English translation.

4. A. F. Kalinkevich, Zemledelie No. 6, 45 (1954).
5. E. M. Eaton and D. R. Ergle, Plant Physiol. 28, 3, 503 (1953).
6. A. A. Borodulina and V. I. Runov, Physiological Basis for Foliar Nutrion of Plants [in Russian] (Tashkent, 1959).
7. S. S. Serbin, Agrobiologia, No. 5, 89 (1954).
8. F. F. Matskov, Foliar Nutrition of Plants [in Russian] (Kiev, 1957).
9. B. L. Dorokhov, Effect of Foliar Feeding on the Rate of Photosynthesis. Author's Abstract of Candidate's Dissertation [in Russian] (Kiev, 1957).
10. A. P. Kalashnikov. V. O. Chistov, S. I. Lipman, and A. P. Kalashnikova, Vestnik Sel'khoz Nauki, No. 5, 142 (1957).

EFFECT OF MICROELEMENTS ON COLD RESISTANCE OF CORN

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The introduction of corn into the northern regions raises the important problem of increasing its cold resistance. After considering the data available in the literature [1-4, and others] concerning the capacity of some microelements – aluminum, copper, zinc, manganese, molybdenum – to increase the frost resistance of plants, we have proposed that these microelements are also able to increase the cold resistance of plants. In order to check this hypothesis, we began experiments in 1955-1956, in the V. L. Komarov Botanical Institute, Academy of Sciences of the USSR, to study the effect of a one-day preplanting treatment of seeds with solutions of microelements on the germination of corn seeds at various planting times, and therefore under various temperatures; we also began experiments to study the effect of preplanting treatment of seeds with microelements on the cold resistance of young shoots during short-duration action of temperatures close to 0°.

There are data in the literature [5,6,7, and others] about the fact that treatment of the initiated seeds of high-temperature plants with constant low temperature (0, -1.2, and even -5°) for 10, 15, 30 days prior to planting, or alternation of low temperatures (0, -10°) with high temperatures (17-19°) in a ratio of 12 hours: 12 hours or 6 hours: 18 hours, causes an acceleration of seed germination during lower temperatures, increases the cold resistance of plants, accelerates development, and increases yield.

It was of interest to compare the effect of preplanting treatment of seeds with microelements with the effect of constant low or alternating temperatures on the germination of maize seeds at lower temperatures, and also to clarify whether it is possible, with the aid of preplanting seed treatment with microelement solutions in combination with the other methods of seed treatment, to increase the effect of plant resistance to lowered temperatures.

Experiments On The Effect Of Microelements On Corn Seed Germination At Various Temperatures

Experiments of 1955

After the first planting – May 24 – there was stable below-normal temperature for a long time; for the first five-day period it was 6.2°, the second, it was 11.1°, and the third, 12.2°. These figures indicate that the temperature conditions were very unfavorable for corn seed germination, especially during the first five days. Under these conditions (during the lowered soil temperature), the interesting fact of a considerable speeding up of corn seed germination under the effect of the microelements manganese, zinc, molybdenum, and especially copper, showed up, as well as a considerable increase in the percent of seed germination under their influence. The latter is especially important, since the lower temperatures considerably decrease the percentage of germination. Boron did not show any positive effect, either on the speed of germination or on the percent of germination; on the contrary, it even had a negative effect on seed germination under these conditions. This is illustrated in Fig. 1A.

Our data on the acceleration of germination at lowered temperatures, obtained when corn was treated with copper, agree with the data of Abaeva [8], obtained with cotton. However, in our experiments it was possible to show that in addition to copper, zinc, molybdenum, and manganese give almost similar effects.

Germination in the second period of planting (Fig. 1B) occurred under more favorable temperature conditions. The average daily temperatures were high, averaging 12°. This conditioned the appearance of stronger sprouts in all treatments, both in controls and microelement treatments. It is true that in this experiment, too, certain microelements (zinc, manganese, copper) also showed a positive effect on the speed of germination, but this effect was considerably less than in the first experiment.

Experiments of 1956

The results obtained in the experiments of 1956 completely confirmed the data of 1955 (Table 1).

As can be seen from Table 1, the seeds treated with copper and zinc germinated on the 12th day, those treated with molybdenum, on the 14th day, while control plants and those treated with boron germinated only on the 18th day. As in 1955, copper, zinc, and molybdenum considerably increased the percent of germination.

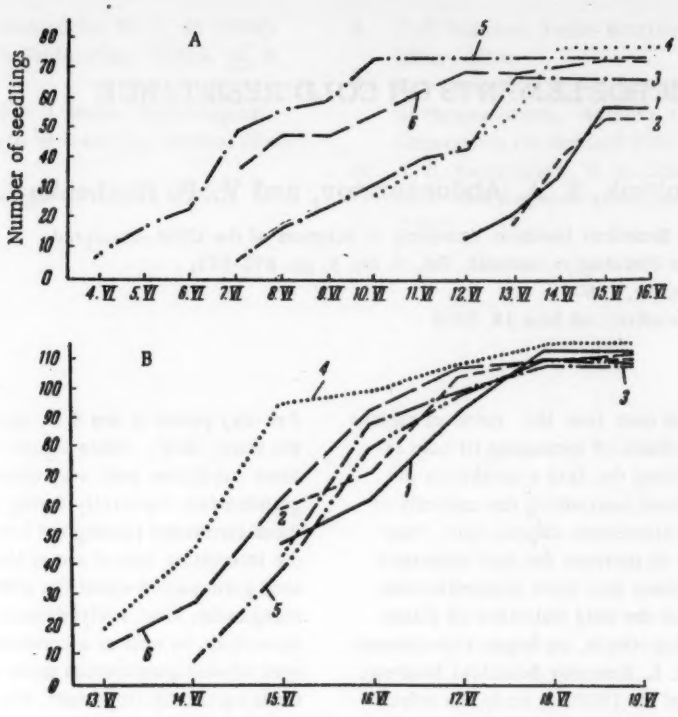

Fig. 1. Effect of microelements on germination of corn seeds after planting in cold soil (A) and in warmer soil (B). 1) Control; 2) boron; 3) molybdenum; 4) manganese; 5) copper; 6) zinc.

TABLE 1. Effect of Microelements on Seed Germination in Corn in Lowered Temperatures (Planted May 8; average temperature up to the appearance of shoots was 8 to 9°.)

Experimental treatment	No. of germinated seeds out of 100 planted										No. of days up to appearance of seedlings	
	May						June				first seedlings	mass seedlings
	20	22	24	26	28	30	1	3	5	7		
Control (dry)	—	—	—	22	34	37	45	56	57	60	18	26
Control (wet with water)	—	2	3	13	18	24	33	46	47	48	18	26
H_3BO_3-0.2 g/liter	—	1	3	18	32	35	39	51	51	51	18	26
$(NH_4)_2MoO_4$-0.25 g/liter	—	9	28	50	61	67	68	69	69	69	14	22
$MnSO_4$-0.5 g/liter	1	4	6	31	55	60	62	63	63	63	16	22
$CuSO_4$-0.04 g/liter	13	32	57	77	77	79	81	81	81	81	12	18
$ZnSO_4$-0.2 g/liter	16	27	46	65	75	76	76	78	78	78	12	20

In 1956, the considerably lesser positive effect of microelements on germination at higher temperatures (planted May 22 and June 6) was also confirmed.

The hardening of seeds with constant low tempertures did not show any positive effect on germination in cold soil (average daily temperature 9.8°); it even decreased the percent of germination. The hardening with alternate temperatures, however, had some positive effect. In all cases, both when preplanting seed hardening was combined with preplanting seed treatment with microelements, as well as with microelement treatment alone, the microelements – copper, zinc, manganese, and in some cases, molybdenum – considerably increased the speed of germination and percent of germination at

TABLE 2. Effect of Preplanting Hardening of Seeds by Alternating Temperatures and their Treatment with Microelements

Experimental treatments	No. of germinated seeds out of 50 planted										No. of days from planting to appearance of seedlings	
	May					June					1st seedlings (not less than 5)	mass seedlings
	27	28	29	30	31	1	2	3	4	5		
Alternating temperatures in combination with treatment by microelements												
Control	3	6	18	22	34	34	38	38	38	38	12	15
H_3BO_3 – 0.2 g/liter	1	6	15	24	30	32	37	37	38	38	12	15
$(NH_4)_2MoO_4$–0.25 g/l	2	11	23	31	38	38	38	38	38	38	12	14
$MnSO_4$—0.5 g/liter	2	8	23	24	36	38	39	39	39	39	12	15
$CuSO_4$—0.04 g/liter	13	19	34	37	41	41	43	43	43	43	11	13
$ZnSO_4$—0.2 g/liter	9	16	24	35	37	38	38	38	39	39	11	14
Treatment only with microelements												
Control	—	2	6	9	23	33	34	36	36	36	13	18
H_3BO_3-0.2 g/liter	1	3	5	12	21	31	39	39	39	39	13	18
$(NH_4)_2MoO_4$-0.25 g/l	—	3	14	18	29	36	37	38	38	38	13	17
$MnSO_4$-0.5 g/liter	2	9	12	34	43	43	43	43	43	43	12	16
$CuSO_4$- 0.04 g/liter	7	13	15	29	39	39	39	39	39	39	11	16
$ZnSO_4$-0.2 g/liter	11	14	15	24	38	40	40	40	41	41	11	16

TABLE 3. Effect of Microelements on Quantitative Content of Glutamic Acid and Serine in Corn Embryos at Various Temperatures of Germination (in μg N of given amino acid)

Experimental treatment	Glutamic acid	Serine	Glutamic acid	Serine
Expt. No. 1	At 7.5° for 48 hours		At 21° for 48 hrs	
Control	less than 0.1	1.18	1.22	2.27
Aluminum	0.59	1.48	1.28	3.30
Copper	1.18	1.78	1.38	3.16
Cobalt	1.28	1.47	0.89	2.07
Expt. No. 2	At 12.5° after 24 hours		At 16° after 24 hrs	
Control	0.66	3.63	—	3.63
Aluminum	0.99	4.95	—	3.96
Copper	1.65	5.44	—	4.95
Cobalt	1.66	5.11	—	4.62
Expt. No. 3	At 7.5° after 18 days		At 10° after 9 days	
Control	1.98	4.62	3.13	4.04
Aluminum	4.62	5.94	3.79	4.12
Copper	3.96	6.27	4.12	4.15
Cobalt	2.97	4.95	3.63	5.19

lowered temperatures (Table 2). These data thus indicate the advantage of preplanting seed treatment with microelement solutions as compared with the more elaborate method of seed hardening with alternating temperatures.

We have also obtained data on a positive effect on maize seed germination at lower temperatures (7.5-9°) obtained through preplanting treatment of seeds in solutions of aluminum.

It is known that in germinating seeds protein hydrolysis is actively occurring, with formation of free amino acids, which then are used for the nutrition of the developing embryo and for construction of the tissues of the young plant. It was of interest to study the effect of

TABLE 4. Effect of Microelements on Cold Resistance of Corn

Experimental treatments	State of plants after treatment with lowered temp.											Air-dry weight of 10 plants	
	No. of plants in which injuries were found						No. of plants after a month						
	1 leaf		2 leaves		3 leaves								
	weakly	strongly	weakly	strongly	weakly	strongly	noninjured	dead	weakly injured and recovered	strongly injured and not recovered	height of plants, cm	grams	in % of control
Control	4	0	2	6	2	11	0	11	6	8	7	1.83	100
H_3BO_3 —0.2 g/liter	6	0	3	8	3	5	0	5	9	11	10	2.65	139
$(NH_4)_2$ MoO_4— 0.25 g/liter	8	0	3	2	1	1	10	1	11	3	13	3.84	209
$MnSO_4$ —0.5 g/liter	5	0	7	5	1	5	2	4	12	7	10	2.82	154
$CuSO_4$ — 0.04 g/liter	6	0	4	3	0	3	9	2	10	4	12	3.21	175
$ZnSO_4$ —0.2 g/liter	4	0	3	3	1	2	12	0	7	6	13	3.60	197

Note: There were 25 plants in the experiment.

microelements on the composition and quantitative content of free amino acids in maize seeds germinating at various temperatures. This was studied by means of paper chromatography. It turned out that the amount of amino acids is small in the embryos of germinating seeds at lowered temperature (7.5°) and in control seeds (seeds treated with water), while in those treated with aluminum, copper and cobalt, the amount is considerably greater. On germination of seeds at higher temperature (21°), no difference is observed in the free amino acid content of the embryos of control seeds and those treated with microelements. These same results were obtained with the free amino acid content of the endosperm of corn seeds, the content and composition of which was considerably lower than in the embryo.

The data on the quantitative content of glutamic acid and serine (Table 3) also confirm what was said about the considerable increase in free amino acid content in the embyro under the influence of microelements, especially at lowered temperatures (from 7.5 to 10°).

The increase in hydrolytic breakdown of proteins in the embryos of germinating corn seeds under the influence of microelements at lowered temperatures is evidently connected with their capacity to increase the activity of proteolytic enzymes at these temperatures.

Experiments On The Effect Of Microelements On Cold Resistance Of Corn Sprouts

For the study of the effect of microelements on the resistance of sprouts to lowered temperatures, two experiments were carried out in triplicate in 1956. The first was begun June 21, the second, on July 28. The seeds, previously treated with the microelement solutions, were planted in sand in clay pots, to which Helriegel nutrient mixture was applied. The plants were subjected to the cold treatment at the stage of 3 to 5 leaves.

The data on the effect of the microelements boron, molybdenum, manganese, copper and zinc on frost and cold resistance of maize sprouts are given in Tables 4 and 5. In Table 4 are given the results of the first experiment started on June 21, in which maize plants at the stage of 3 leaves were transplanted, without previous hardening with lowered temperatures, from the greenhouse, where the temperature was up to 26°, into the refrigerator at a temperature of 0 to −0.5° for 3 hours. The injury to the plants was recorded at 3 days and after a month of low temperature action.

As can be seen in Table 4, the control plants were most injured by the short-duration treatment with low temperatures. The greatest increase of frost resistance was obtained in treatments with zinc, molybdenum, and copper. Manganese and boron showed a lesser positive effect.

In Table 5 are given the data of the second experiment, started on July 28. In view of the data present in the literature [1,10] concerning the positive action of aluminum on frost resistance of plants, the effect of various doses of aluminum sulfate and aluminum chloride were also studied in this experiment. On August 22, the plants of this experiment at the 5-leaf stage were placed in the refrigerator, where the temperature was 3 to 4°, for 48 hours. Thus, in this experiment we were concerned with the effect of microelements not on frost resistance but on cold resistance. The growth of plants, prior to the cold treatment, took place at lower temperatures than in the first experiment; the average daily temperature in August was only 13.9°; at night it often dropped to 7°, which created conditions of a certain hardening of plants, as distinguished from the first experiment.

As can be seen from the data given in Table 5, all microelements studied, especially aluminum, copper,

TABLE 5. Effect of Microelements on Cold Resistance of Corn Seedlings

Experimental treatment	State of plants after a month of treatment with lowered temp.									
	no. of plants				height of plants, cm		air-dry weight of plants			
							treated with lowered temp.		not treated with lowered temp.	
	noninjured	dead	completely recovered	not recovered	treated with lowered temp.	not treated with low temp.	grams	% of control	grams	% of control
Control	3	5	3	4	14	18	1.52	100	4.11	100
H_3BO_3 — 0.2 g/liter	5	1	3	6	14	21	1.84	121	4.83	107
$(NH_4)_2$ MoO_4 — 0.25 g/liter	8	0	5	2	18	23	2.50	184	5.34	129
$MnSO_4$ — 0.5 g/liter	5	0	6	4	16	28	2.51	155	6.02	146
$CuSO_4$ —0.04 g/liter	11	0	4	0	20	26	3.66	241	5.60	136
$ZnSO_4$-0.2 g/ liter	10	0	4	1	21	25	3.76	247	6.10	148
$Al_2(SO_4)_3$-10 g/l	10	0	5	0	22	—	3.98	261	—	—
Same 20 g/liter	13	0	2	0	23	—	4.75	312	—	—
" 50 g/liter	10	0	5	0	20	—	4.21	276	—	—
$AlCl_3$ —10 g/liter	9	0	6	0	21	—	4.02	264	—	—
Same —20 g/liter	11	0	4	0	19	—	4.13	272	—	—
" —50 g/liter	2	6	4	3	11	—	1.28	91	—	—

Note: There were 15 plants in the experiment.

and zinc, increased the cold resistance of corn sprouts. The highest dose of aluminum chloride turned out to be toxic. All microelements, especially aluminum, copper, zinc, and molybdenum, increased the yield of aerial-dry mass of shoots considerably more in those cases where plants were subjected to treatment with lowered temperatures than if they were not subjected to such treatment. In the case of the optimal dose of aluminum, a three-fold increase in yield was observed.

SUMMARY

Microelements are able to increase not only frost resistance, as has already been pointed out in a number of the papers cited, but also the cold resistance of plants.

The data obtained in the experiments show the prospects offered by the method of preplanting treatment of seeds in microelement solution, when corn is introduced into the northern regions.

LITERATURE CITED

1. L. I. Sergeev and K. N. Sergeeva, Doklady Akad. Nauk SSSR 22, 9, 535 (1939).
2. M. Ya. Shkol'nik, Éksp. Botanika, Ser. IV, 10, 228 (1955).
3. M. M. Okuntsov and M. N. Sileva, Uch. Zap. Tomsk. Univ. 13, 7 (1950).
4. A. G. Mikhalovskii and M. M. Sopil'nyak, Sov. Agronomya, 3, 70 (1954).
5. E. A. Grachev, Trudy, Vol'no-ékonomich. Obshchestva 1, No. 2 (1875).
6. A. E. Voronova, Dostizh. Nauki i Peredov. Optya v Sel'skom Khozyaistve, No. 4, 69 (1953).
7. P. A. Genkel', A. P. Sarycheva, and O. A. Sitnikova, Fiziol. Rastenii 2, 447 (1955).
8. S. S. Abaeva, Microelements in Agriculture and Medicine [in Russian] (Izd. Latvian SSR, 1956).
9. N. Cojeneanu, Acad. R. P. Romane 3, No. 3 (1953).

THE USE OF LEAF DIAGNOSTICS OF THE NUTRITION OF SUMMER WHEAT

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When the method of leaf diagnostics was first being used, D. A. Sabinin, in evaluating the first works of the French scientists Moma and Lagateu, wrote: "The method of leaf diagnostics makes it possible for us to overcome to a significant degree the empiricism of the usual field experiments with fertilizers" [1]. D. A. Sabinin expressed great confidence in the future method of diagnosing the demands of plants for fertilizers by physiological indicators as a basic tool of agricultural chemistry control in agriculture.

At the present time, analysis of the leaves is so widely used in diagnosing plant demands for fertilizer that the creation of a job of leaf diagnostics is a goal outside of our borders [2]. Thus, the application of mineral fertilizers under oil crops [3] in France is done with the calculation of data of the usual chemical analysis of the leaves.

In our country, methods of quick chemical analysis of the sap extracted from tissues or reactions on sections obtained a large dissemination [4-6, and others]. Meanwhile, the diagnosis of nutrition by the usual chemical analysis of leaves, which is more exact and sufficiently quick with modern methods of laboratory analysis, still has not obtained the proper development.

It is necessary to establish "critical levels" of the concentration of nutrient elements in the leaves and optimal (equilibrium) relationship between the elements for the use of data of leaf analysis to determine the needs of plants for mineral fertilizers. Composite data on this question are presented in works concerning a majority of fruit and technical crops [7-11, and others].

The development of methods of leaf analysis applicable to grass crops, and also a physiological basis of the method of leaf diagnostics, was made in a number of work [12-15 and others]. In our investigations carried out in 1954-1959 on chernozems in Omsk oblast, critical levels were established for the nutrition of summer wheat, which correspond to the concentration in the leaves at the end of vegetation of 2.6-3.0% nitrogen, 0.52-0.60% P_2O_5 and approximately 2.8-3.0% K_2O.

In addition to the percent concentration of nutrient substances characterizing the quantitative side of nutrition and its intensity, the relationship between the elements in the leaves, or the qualitative side of nutrition, is also significant.

Many facts were accumulated in the literature on diagnosis which suggest that equilibrium nutrition of a crop is a specific optimal relationship between the nutrient elements in the leaves [3,12,15f] and in the entire plant [16-18]. This optimal relationship between nitrogen and phosphorus in the leaves shortly before flowering or at the time of flowering varies from 12 to 16 (from 5.2 to 7 on conversion to P_2O_5) and is encountered in plants of the most remote families. The physiological side of this effect has not yet been studied sufficiently.

On the basis of our numerous experiments, it was established that the equilibrium nutrition of summer wheat can be expressed by the following equations characterizing the relationship between the percent concentration of nutrient elements in the leaves at the end of flowering:

$$N \approx a^2 \cdot P_2O_5 \approx K_2O^*, \text{ where } a^2 = 5 \text{ (varies from 4 to 6).} \quad (I)$$

If the percent of nitrogen in the leaves at the end of flowering reaches the "critical level" and the relationship between nitrogen and P_2O_5 is greater than 5, then the wheat plants do not need nitrogen and they experience a demand for phosphorus. If this relationship is below 5, then the plants have a surplus of phosphorus and need nitrogen. The degree of the demand of the plants for the element depends, as noted, on how wide or narrow is the relationship between nitrogen and P_2O_5 in the leaves, and also on the percent figures of these elements which do not reach or exceed the "critical level" of nutrition in the leaves. In the presence of the equation (I) and other favorable conditions of the external environment, we established a number of interrelationships between nitrogen and phosphorus in the leaves

* The relationship between K_2O and other elements ($N:K_2O$; $P_2O_5:K_2O$) must in the future be made more precise in view of our few experimental data on potassium.

(*l*) and grain (g) of summer wheat which are expressed in the following equations:

Nitrogen in grain and leaves	$N(g) \approx k \cdot N(l)$, where k is the coefficient of transfer of nitrogen of the leaves into nitrogen of the ripe grain, equal to 0.9-1.0 [15e]	(II)
	or $N(g) \approx 0.85 \cdot N(l) + 0.27$	(IIa)
Nitrogen and phosphorus in the grain:	$N(g) \approx a \cdot P_2O_5(g)$, where $a = 2.25$	(III)
Nitrogen in grain and phosphorus in leaves:	$N(g) \approx a^2 \cdot P_2O_5(l)$, where $a = 5$.	(IV)
Phosphorus in grain and leaves:	$P_2O_5(g) \approx a \cdot P_2O_5(l)$, where $a = 2.25$.	(V)

Equations II-V can be combined into one general equation:

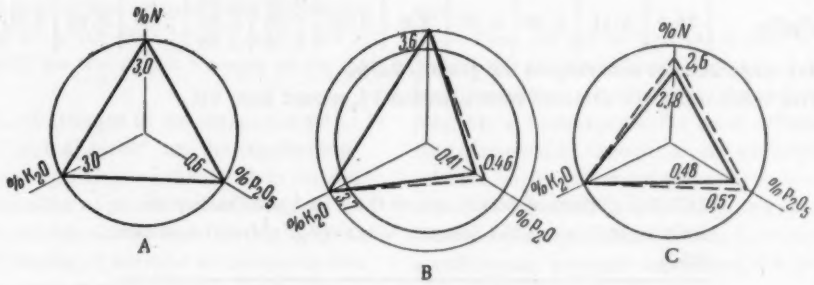
$$N(g) = k\,N(l) \approx a \cdot P_2O_5(g) \approx a^2 \cdot P_2O_5(l) \qquad (VI)$$

These equations (I-VI) together with indicators of the critical level of nutrition make it possible to characterize the quantitative and qualitative sides of plant nutrition, to forecast and explain the action of mineral fertilizers on yield, and also to serve as an estimate of the quality of the grain of summer wheat. This is supported by the results of experiments presented in Tables 1-3 and in Fig 1. The method of establishing the experiments, selection and analysis of the plant samples were described in other work [15e,f].

Chemical analysis of the leaves of wheat grown on a field of State Farm "Élita" showed (Fig. 1A,B) that plants taken from nonfertilized plots and fields had an excessively high level of nutrition with nitrogen and, obviously, with potassium, but had a strongly expressed deficiency of phosphorus. This led to the fact that a clearly expressed disproportion was created in the leaves between the basic protoplasmatic elements (Table 1).

We can see from the data in Table 1 that the relationship between nitrogen and P_2O_5 in the first three indicators exceeded the optimal quantities, which indicates an excess of nitrogen and insufficient phosphorus in the leaves of wheat grown on the fields of State Farms. Therefore, the introduction of nitrogen and potassium fertilizers was hardly reflected in the chemical composition of the leaves (relative concentration of N and K_2O in them increased 3-4%). On the other hand, when superphosphate was applied, an increase was observed in the concentration of P_2O_5 in the leaves on the average for two years of 12% (Fig 1,B). On the basis of the data of leaf diagnostics the conclusion was reached that phosphoric acid fertilizers must show the basic effect on the yield of wheat grain on the inspected fields of State Farms "Élita" and "Boevoi". This prognosis was supported by the data of field [15f] and small plot [15 d,e] experiments carried out on State Farm "Élita" in 1957-1958 (Table 2).

The presence of a disproportion between nitrogen and P_2O_5 in the leaves of wheat on these State Farms was reflected on the formation of the chemical composition of the grain, although the grain, in contrast to the leaf



Leaf diagnostic of condition of nutrition of summer wheat. A) Indicators of the "critical levels" of nutrition of summer wheat; B) type of phosphorus deficiency with excess of nitrogen and potassium for wheat planted on soils of the "Elita" State Farm. Heavy line is condition at the start; dash line is the change obtained from the application of superphosphate; thin line of the circle describes the radius corresponding to 3.6% nitrogen – it is an indicator of the possible increase of the concentration of P_2O_5 in the leaves (up to 0.60-0.72%) for obtaining equilibrium nitrogen, phosphorus and potassium nutrition of summer wheat; C) type of nitrogen deficiency for plants on a small plot on the large experimental field of the Agricultural Institute. Heavy line is the condition at the start; dash line is the change obtained as a result of the application of a complete mineral fertilizer (NPK).

TABLE 1. Interrelationship Between the Basic Protoplasmatic Elements with Equilibrium and Nonequilibrium Nutrition of Summer Wheat

No.	Relationship	Organs analyzed	Figure corresponding to equilibrium nutrition	Figures characterizing nutrition conditions for Mil'turum 553 wheat on chernozems: state farm "Elita", 1957-1958	state farm "Boevoi", 1959	exptl. farm no. 1, Omsk agricul. Institute
1	$N : P_2O_5$	Leaves	5 (a^2)	8.8	11.5	4.5
2	$N : P_2O_5$	Grain	2.25 (a)	3.1	3.34	2.27
3	$\frac{N}{P_2O_5}$	$\frac{\text{Grain}}{\text{Leaves}}$	5 (a^2)	6.3	7.69	5.4
4	$\frac{N}{N}$	$\frac{\text{Grain}}{\text{Leaves}}$	0.90—1.0 (κ)	0.75	0.70	0.90—1.0
5	$\frac{P_2O_5}{P_2O_5}$	$\frac{\text{Grain}}{\text{Leaves}}$	2.25 (a)	2.02	2.35	2.38
6	$N : K_2O$	Leaves	1	1	—	—

TABLE 2. The Action of Mineral Fertilizers on the Yield and Quality of the Grain of Mil'turum 553 Summer Wheat (State Farm "Elita", 1958).

Experimental variant	Grain yield, centners/ha	Conc. in leaves at end of flowering as % of absolutely dry substance: N	P_2O_5	K_2O	$N:P_2O_5$	Comp. of grain in % of abs. dry substance: estimate: N	P_2O_5*	$N:P_2O_5$*	actual: N	P_2O_5	$N:P_2O_5$
Control	19.9	3.73	0.49	3.82	7.6	2.80	1.10 1.00	2.54 2.80	2.75	0.85	3.24
N_{30}	19.6	3.73	0.44	—	8.5	2.80	0.99 0.96	2.83 2.92	2.90	1.00	2.90
P_{60}	25.9	3.63	0.47	—	7.7	2.72	1.06 0.98	2.56 2.78	2.86	1.02	2.80
$N_{30}P_{60}$	25.8	3.81	0.45	3.73	8.5	2.86	1.01 0.99	2.83 2.89	2.81	0.93	3.02
$N_{60}P_{60}K_{30}$	26.1	4.11	0.48	4.10	8.6	3.08	1.08 1.04	2.85 2.96	2.89	0.94	3.08

Note: Experimental accuracy on the grain is 2.5%.

* First series of figures obtained from equation VI, second from VII.

TABLE 3. Effect of Fertilizers on the Yield and Quality of Grain of Mil'turum 553 Summer Wheat (Agricultural Institute, 1956).

Experimental variant	Grain yield, centners/ha	± m	Nitrogen as % of abs. dry weight: in leaves at end of flowering	in grain
Control	22.2	0.92	2.08	2.00
N_{60}	28.5	0.61	2.00	1.90
$N_{60}P_{60}$	30.5	0.73	2.28	2.15
$N_{30}P_{60}K_{45}$	27.7	1.46	2.10	2.06
$N_{60}P_{60}K_{45}$	29.6	1.39	2.57	2.32
$N_{90}P_{60}K_{45}$	31.1	1.38	2.89	2.65

Note: Fertilizers were applied on the plot in 1955, but because of severe drought their action in the year of application was low.

apparatus, reacted less sensitively to a change in the nutrition conditions.

The relationship between the elements in the grain and in the leaves on calculation of the data in Table 1 can be expressed in the following equation:

$$N(g) : P_2O_5(g) \approx \sqrt{N(l) : P_2O_5(l)} \quad \text{(VII)}$$

This equation sufficiently well expresses the effect of the relative concentration of nutrient elements in the leaves on the chemical composition with conditions of normal or excess nitrogen nutrition of summer wheat. In these cases equation (VII) can be used for checking the accuracy of the estimate of the concentration of phosphorus carried out according to the regular equation (VI).

When using equation (VI) and (VII) for estimates of the concentration of phosphorus in the grain, one must consider the following features of plant nutrition. If the concentration of nitrogen in the leaves is close to the indicated "critical levels" of nutrition for the element, then the estimate of the concentration of phosphorus can be carried out on both parts (left and right) of equation (VI) or by equation (VII). With this the estimate of the concentration of nitrogen in the grain is carried out according to the usual coefficient of transfer [15e]. If the concentration of nitrogen and P_2O_5 in the leaves is higher than the "critical levels" of nutrition, but the relationship between them is in the ranges of optimal (from 4 to 6), then the more accurate estimate of the concentration of phosphorus is by the left part of equation (VI).

With the presence of a strong disproportion between these elements in the leaves to the side of a significant predominance of nitrogen over phosphorus, the right part of equation (VI), that is, equation (V), and also equation (VII), are used for an estimate of the concentration of phosphorus in the grain. In the given case, lower coefficients of transfer of nitrogen of the leaves into nitrogen of the grain are used [15e].

If the percent of nitrogen in the leaves is significantly lower than "critical level", but the relationship between nitrogen and P_2O_5 is lower than 4, then the estimate of the concentration of phosphorus in the grain is made according to the left side of equation (VI). With this, the coefficients of transfer of nitrogen of the leaves into nitrogen of the ripe grain is nearly always greater than unity and ranges from 1.1 to 1.2.

Thus, with the presence of nonequilbrium plant nutrition, that is, with a relationship between N and P_2O_5 greater than 6 or less than 4, that part of equation (VI) where the element whose concentration in the leaves drops below the "critical level" of nutrition is located is used for an estimate of the concentration of phosphorus in the grain. We show this with the data in Table 2.

The figures in Table 2 show that wheat had nonequilibrium nutrition because of a phosphorus deficiency, in connection with which the coefficient of transfer (K) equal to 0.75 was used for an estimate of the concentration of nitrogen in the grain [15e]. We put the calculated concentration of nitrogen in the grain and data of the chemical analysis of the leaves in equations (VI) or (VII). The calculations showed that the size of the concentration of phosphorus and also the relationship between nitrogen and P_2O_5 in the grain, obtained from the right part of the equations (VI) and (VII), is close to the actual data (Table 2). A sufficiently accurate estimate of the concentration of nitrogen and phosphorus in the grain was obtained in all of the three-year field experiments on State Farms and also in vegetation experiments in 1958 and 1959. The error in the estimate of concentration of P_2O_5 in the grain rarely exceeded 10% of its actual concentration.

The use of leaf diagnostics on the soil of a small plot of the Agricultural Institute made it possible to establish that plants were in equilibrium between nitrogen and phosphorus nutrition prior to the application of fertilizers, but the percent of nitrogen in the leaves was significantly lower than the "critical level", and it closely approached equilibrium for phosphorus (Fig 1, C and Table 1). This showed the stronger need of wheat on this plot for nitrogen fertilizer. The application of ammonium nitrate in a dose of 60 kg of grain per hectare in conditions of a wet year greatly stimulated the growth of wheat and increased the yield of grain, but the chemical composition of the leaves and grain for this variant hardly differed from the control (Table 3).

An increase in the dose of nitrogen in the full fertilizer up to 90 kg per hectare aided in reaching the "critical level" of nitrogen in the leaves and enabled us to obtain the highest yield of grain with good quality.

The action of phosphorus on this soil was less great, which is fully explained from the data of the leaf analysis.

Thus, the use of "critical levels" of nutrition together with the established relationships between the basic protoplasmatic elements (N and P_2O_5) made it possible: a) to determine the basic deficiency in nutritive elements; b) to forecast and explain the action of mineral fertilizers on the grain yield; c) to spread the results of experiments with fertilizers obtained on one field to other agricultural fields; d) to put into practice a sufficiently accurate estimate of the quality of grain of summer wheat.

This suggests that leaf diagnostics not only explain the results of the field experiments, but also raise the empirical experimentation to the level of the scientific estimate.

LITERATURE CITED

1. D. A. Sabinin, Khimizatsiya Sots. Zemled. No. 1, 50 (1932).
2. V. V. Tserling, Pochvovedenie, No. 3 (1957).
3. P. Prevo and M. Ollan'e Fiziol. Rastenii 3, 554 (1956).
4. G. S. Davtyan, Khimizatsiya Sots. Zemled. No. 3, 68 (1934).

5. K. P. Magnitskii, Field Control of Plant Nutrition [in Russian] (Izd. "Znanie," 1958).
6. V. V. Tserling, Udobrenie i Urozhai, No. 7, 25 (1958).
7. C. E. Beauchamp, Plant Physiol. 17, 165 (1942).
8. W. Thomas, Soil Sci. 59, 5, 353 (1945).
9. A. Ulrich, Plant Physiol. 3, 207 (1952).
10. W. Reuther and P. F. Smith, Analyse de plantes et problémes des engrais minéraux (I.R.H.O., Paris, 1954) p. 166.
11. C. Bould, Analyse de plantes et problémes des fumures minerales (I.R.H.O., Paris, 1956) p. 299.
12. H. Lundegardh, Leaf Analysis (London, 1951).
13. J. Dulac, Compt. rend. Academie d'agriculture de France 41, 7, 328 (1955).
14. E. H. Tyner, Soil Sci. Soc. Am. Proc. 11, 317 (1947).
15. N. K. Boldyrev, a) Sel'. khoz. Sibiri, No. 2, 44 (1957); b) Doklady Akad. Nauk SSSR 119, 1, 171 (1958)†; c) Fiziol. Rastenii, 6, 73 (1959)†; d) Doklady Akad. Nauk SSSR 126, 4, 886 (1959)†; e) Pochvovedenie, No. 11, 104 (1959); f) Sel'. Khoz. Sibiri, 10, 12 (1959).
16. A. Yu. Levitskii and A. A. Lesyukova, Khimizatsiya Sots. Zemled., No. 2 (1935).
17. I. Kolarik, Za Sotsial. Sel. Khoz. Nauku A5, 3, 332 (1956).
18. I. Kolarzhik, Mezhdunarodnyi Sel. Khoz. Zhur. No. 2, 43 (1958).

†See English translation.

INCREASE IN PHYSIOLOGICAL ACTIVITY AND YIELD OF FRUIT TREES WITH FOLIAR FEEDINGS

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In recent years in both the USSR and abroad a great number of papers have appeared pointing out the increase in yield of apple trees after foliar feedings [1-8].

There is less information concerning the efficiency of foliar feedings on stone fruits.

In one of the investigations that has been carried out with fruit trees, the chief attention of the investigators was directed to the effect of foliar feedings on yield and quality of the crops produced. The physiological-biochemical picture of the effect of foliar feedings on plants is extremely complex and as yet has not been sufficiently studied. The use of fertilizer by plants produces great shifts in metabolism and, consequently, in the chemical composition of the plant under the effect of changing conditions of the external medium. These shifts are very closely connected with changes in the activity of enzyme systems in plants, the most important of which are the oxidation-reduction enzymes. The effect of foliar feeding on the activity of oxidation-reduction enzymes has been insufficiently studied; the data obtained in this field usually involve crops and action of widely varying mixtures in quite incompatible experimental conditions.

However, it is known from the literature that when favorable timing is selected, it is possible by foliar feedings to affect the enzyme apparatus of leaves, particularly the activity of oxidation-reduction enzymes. The work of Vlasyuk and Klimovitskaya [9], who studied the activity of catalase and peroxidase in rye, corn, and sugar beet, has shown that spraying plants with salts has a stimulating action on the enzyme activity of plants. In the experiments of Mashtakov [10], an analogous result was obtained when potatoes were sprayed with superphosphate. An increase in ascorbic acid oxidase activity in oats, cabbage, tomatoes, and other crops after foliar feedings has been noted by Peive and Krauia [11].

We know of no papers concerning the effect of foliar feedings on oxidation-reduction processes in fruit trees.

In the present paper we report the results of our experiments on the effectiveness of foliar feedings in fruit trees under the conditions of the droughty Southeast, and their effect on the activity of a number of oxidoreductases and oxidation-reduction substances. Testing of the method of foliar feedings under drought conditions is of great interest, since with a soil moisture deficit, the substances have difficulty in getting through the roots, and occasionally fertilizers introduced into soil are left unused.

The work has been carried out under the direction of the late Professor N. P. Krasinskii.

METHODS

Foliar feeding was tried out in the course of 1955-1957 on cherry and apple. In 1956 and 1957, two students in the department of plant physiology and microbiology, L. Danchenko and R. Parshina, participated in the work.

In 1955, the experiment was carried out in Uchkhoz of the Saratov Agricultural Institute, and in the Michurin kolkhoz of Voroshilov region in Saratov district, using 39 cherry trees, variety Rastun'ya and 63 apple trees, variety Pepin litovskii. In 1956, freezing of flower buds in the stone crops, because of the very unfavorable winter conditions, as well as periodicity of fruit-bearing in the apple, caused us to transfer the experiment to the orchards in the Sovkhoz Combine in Saratovskaya region. As experimental plants, 99 apple trees variety Anis Serii and Mal't bagaevskii and 291 cherry trees, variety Lyubskaya and Vladimirskaya, were used.

In 1957, the experiments were again carried out in Uchkhoz of the Saratov Agricultural Institute using Rastun'ya cherry and Pepin litovskii apple.

The trees for the experiments were selected according to their height, degree of flowering, and degree of resistance to unfavorable winter factors. Spraying was carried out in 1955 and 1957 by automakses and in 1956 by a truck sprayer during three periods (approximately at 2-week intervals) – the end of flowering, the stage of young embryos, and the stage of young fruits. The nutrient solution for feeding was prepared by Professor N. P. Krasinskii and was composed of superphosphate – 50 g, ammonium nitrate – 2.5 g, potassium chloride – 2 g, boric acid – 0.5 g (per liter). The pH of the solution was 3. Control trees were sprayed with water. Five li-

ters of solution was used per tree in the case of apple trees, and 3 to 4 liters for cherry trees.

The yield was collected with the aid of the agricultural workers. The quality of the yield was characterized according to the size of the fruit, its sugar content (according to Bertran) and total acidity.

As indicators of the physiological-biochemical activity of the leaf we used: the value of total oxidizability of cell contents (according to Krasinskii); activity of the enzyme dehydrases (Thunberg method), catalase (Bakh method), peroxidase, polyphenol oxidase, ascorbic acid oxidase (method of Povolotskaya and Sedenko). Of the oxidation-reduction systems, we determined reduced ascorbic acid (according to Prokoshev) and glutathione (more precisely, reducing substances titrated with KIO_3). The pH of the cell sap was determined with a potentiometer. Eighty to 100 leaves were cut at 7 to 8 a.m. on the eighth day after the second spraying and on the 8th and 21st days after first spraying.

The selection of the time of sampling was not accidental. We have found that the strongest reaction of leaves to the foliar feeding was obvious in the first 7 to 9 days after spraying. Similar facts were also noted by Yakovleva [12].

However, after the first spraying, the increased enzyme activity (in relation to the water control) is in evidence only for a few days, later falling to the level of enzymatic activity in control leaves. At the second and third spraying, the increased reaction of leaves is retained considerably longer, and even after the subsequent decrease it is higher than in that part of the trees which were not fed aerially.

TABLE 1. Effect of Foliar Feeding on Activity of Dehydrases and Catalase in Leaves of Cherry and Apple.
Note. In this and following tables I, II, and III are times of analysis: I) after second spraying; II) after third spraying; III) on 21st day after third spraying.

Crop, variety	Experimental treatment	Activity of dehydrases (method of decoloration of methylene blue, in min)			Activity of catalase (in cc. 0.1 N $KMnO_4$ per g dry weight)		
		I	II	III	I	II	III
Apple Pepin litovskii	Foliar feeding	23	15	28	11.1	16.4	9.3
	Control	34	25	33	6.2	7.9	5.9
Cherry Rastun'ya	Foliar feeding	—	—	—	22.4	23.3	13.8
	Control	—	—	—	19.5	21.3	10.4

EXPERIMENTAL RESULTS

The data obtained on the activity of dehydrases and catalase in the leaves of apple and cherry are given in Table 1.

As can be seen in the data of Table 1, the dehydrase activity in the apple leaves is high. But the regularity of increase of enzymatic activity after aerial feedings is clearly shown in dehydrases and in catalase – the tendency which is kept for 21 days after the last spraying.

Catalase activity in the leaves of both treatments decreases toward the end of growth, while the dehydrase activity does not change significantly. It is possible that the catalase mechanism of oxidation is replaced by the peroxidase type of oxidation toward the end of the growing season.

The increase of catalase activity in relationship to aerial feedings has been noticed by a number of authors in various agricultural crops [9,10].

After foliar feedings the activity of ascorbic acid oxidase, peroxidase, and polyphenol oxidase is changed (Table 2).

From the data of Table 2 it follows that the most active of the oxido-reductases studied in apple leaves is peroxidase; then follows polyphenol oxidase, and finally ascorbic acid oxidase, which has the least amount of activity.

Foliar feeding has a definite effect on oxidase activity. Thus, in all determinations in the leaves of the apple variety Mal't bagaevskii, ascorbic acid oxidase, polyphenol oxidase, and peroxidase activity is higher in the treatment with foliar feedings than in the leaves from control trees. However, in the leaves of variety Pepin litovskii peroxidase activity is increased after spraying and polyphenol oxidase activity is decreased, as compared with analogous samples from the control trees.

One of the reasons for lack of correspondence shown by the annual data is evidently the specificity of action of oxidative systems in various varieties. Thus, the

TABLE 2. Relationship Between Activity of Ascorbic Acid Oxidase (A) Peroxidase (B) and Polyphenol Oxidase (C) in Leaves of Apple and Cherry, and Foliar Feedings (in mg of ascorbic acid per g of dry weight)

Crop, variety	The year of experiment	The time of analysis	Experimental treatment					
			foliar feeding			control		
			A	B	C	A	B	C
Apple Mal't bagaevskii	1956	I	2.54	8.70	48.90	1.95	2.14	38.25
		II	2.46	8.60	51.20	1.59	2.14	38.56
		III	3.01	10.48	55.20	2.03	8.13	54.04
Pepin litovskii	1957	I	—	3.20	49.08	—	3.75	38.41
		II	—	5.76	60.03	—	7.0	42.27
		III	—	2.81	38.23	—	3.37	34.21
Cherry lyubskaya	1956	I	2.25	0.76	55.38	0.17	2.03	36.42
		II	1.35	0.73	30.80	0.73	2.14	28.90
		III	2.67	1.61	39.41	2.13	1.84	32.65
Rastun'ya	1957	I	—	2.20	65.00	—	3.31	55.65
		II	—	3.12	51.38	—	—	34.33
		III	—	2.44	35.76	—	7.98	22.93

TABLE 3. Effect of Foliar Feeding of Apple, variety Pepin litovskii, on Content of Ascorbic Acid, Iodine-reducing substances, and General Oxidizability of Cell Content in Leaves (per g dry weight)

The time of analysis	Ascorbic acid content, in mg %		Content of iodine-reducing substances mg %		General oxidizability of cell content, ml 0.1 N $KMnO_4$	
	foliar feeding	control	foliar feeding	control	foliar feeding	control
After second spraying	183.5	168.6	4.60	3.07	280.0	214.9
After third spraying	205.1	173.2	5.00	4.85	299.1	216.2
On 21st day after third spraying	200.4	189.4	5.31	4.97	291.2	230.1

study of enzyme activity by Rubin and Sisakyan [13,14] in apple has shown a close dependence on the variety. In the early apple varieties Kitaika zolotaya and Grushanka, the leaves have shown low peroxidase activity, approaching zero; while in the late varieties Pepin litovskii and Slavyanka, peroxidase was active.

In leaves of cherry varieties Rastun'ya and Lyubskaya, the distribution of enzyme activity was found to be analogous to that of the leaves of the apple Pepin litovskii, i.e., during all the time of analysis the leaves from the trees receiving supplementary feeding had more active peroxidase and less active polyphenol oxidase as compared to the leaves of trees which were sprayed with water.

It is interesting to compare results obtained in our experiments with the data of Shakhov [15]. That author has found in many crops (oleaster, hops, hawthorn, chokecherry, etc.) that an increase in peroxidase activity is observed after pickling, accompanied by a fall in polyphenol oxidase activity. Considering that the foliar feeding of leaves with nutrient salts must at least temporarily increase the salt concentration of protoplasm and cell fat, it can be suggested that its action "in the short run" is comparable to the action of pickling.

From the data of Tables1 and 2 we may draw certain conculsions about the increase of energy in oxidation-reduction processes when influenced by foliar feeding.

Rubin and Artsikhovskaya [16], and Rubin and Lutikova [17] consider that the increase in oxidative enzyme activity, particularly of ascorbic acid oxidase, is accompanied by an increase in synthetic reactions.

The increase in synthetic reactions after foliar feedings is also indicated by the increase in ascorbic acid and iodine-reducing substances in leaves (Table 3). Among iodine-reducing substances determined by this method the chief is glutathione (previously this method was used for determination of glutathione). Because of this, one may suppose that, when iodine-reducing substances increase, the glutathione content is also in-

creased. It is interesting to compare the amount of glutathione after spraying with increase in the activity of catalase and peroxidase (Tables 1 and 2), since Thurlaw [18], for example, believes that glutathione is the self-oxidizing component of the cell, and that hydrogen peroxide is formed during its autooxidation. The hydrogen peroxide formed is either broken down by the catalase present in all tissues (catalase activity is increased after foliar feedings), or it may serve for other oxidative processes (peroxide type of oxidation).

TABLE 4. Effect of Foliar Feeding on Yield in Cherry and Apple

Crop, variety	Yr. of expt.	Experimental treatment	Average yield from a tree, kg	In percents of control
Apple				
Pepin litovskii	1955	Foliar feeding	129.2	132.5
		Control	97.5	100.0
Anis seryi	1956	Foliar feeding	39.1	119.9
		Control	32.6	100.0
Mal't bagaevskii	1956	Foliar feeding	32.3	118.7
		Control	27.2	100.0
Pepin litovskii	1957	Foliar feeding	59.3	128.0
		Control	46.3	100.0
Cherry				
Rastun'ya	1957	Foliar feeding	11.6	178.4
		Control	6.5	100.0
Lyubskaya	1956	Foliar feeding	2.8	135.6
		Control	2.1	100.0
Rastun'ya	1957	Foliar feeding	24.6	121.1
		Control	20.3	100.0

TABLE 5. Count of Fallen Fruit at Various Nutrition of Apple, variety Pepin litovskii (average per tree, 1957)

Month	Foliar feeding			Control		
	kg	wt. % *	No. of fruit	kg	wt. % *	No. of fruit
June	0.595	1.0	43	0.859	1.9	125
July	0.170	0.3	10	0.228	0.5	13
August	2.400	4.0	42	2.600	5.4	65
Total	3.170	5.3	95	3.687	7.9	203

* I. e., weight percent of fallen fruit (of collected yield).

Oxidizability of the cell content characterizes the whole tone of the physiological state of the cell. After aerial feedings (see Table 3), there is a sharp increase in the amount of not-quite-oxidized substances (i.e., substances which can be oxidized by permanganate). The latter also testifies about the activation of synthetic reactions in the leaves after spraying with nutrient salts.

The general increase in physiological activity after aerial feedings has been noted in the yield (Table 4).

After looking at the data of Table 4, one can see that the yield of fruit trees varies greatly with the year. Thus, if in 1955 it was high, in 1956 it was very low. The yield was low in 1956 because of very unfavorable wintering conditions for fruit trees in Saratovskya region in 1955-1956. The small apple yield in 1956 is also explained by the fact that the trees had begun only their second year of fruit bearing at the moment of initiation for the experiment.

The data of Table 4 indicate that the salts we used gave an increase in the yield of apples and cherries, particularly significant in the 1955 yield. The increase in yield is also explained by a decrease in ovary fall under the influence of foliar feedings (Table 5).

Most ovaries fell in June (physiological fall of ovaries) and in August (fall of the ripe fruits). Comparing the ovary fall under various nutrition regimes, it can be seen that spraying with NPK + B considerably decreases such fall. The figures on fallen fruit in June are interesting. Control trees had lost 82 ovaries more than the sprayed trees. The fall of ovaries in July does not vary much with the treatments. The count of fallen fruit in August shows that the apples from the control

TABLE 6. Effect of Foliar Feeding on the Quality of Fruit

Crop, variety	Treatment	Percent sugar (per wet weight)	Acidity (according to the malic acid)	Average size of a fruit, g
Cherry Rastun'ya	Foliar feeding	10.00	1.87	3.1
	Control	9.72	1.92	2.2
Apple, Pepin litovskii	Foliar feeding	8.90	1.43	77.0
	Control	8.20	1.47	93.0

trees are less stable on the tree than the apples on the trees which received three foliar feedings. An analogous conclusion was reached by Rubin and his co-workers [1]. This fact has undoubted practical significance for agriculture.

The analysis of fruits has shown that foliar feeding improves their quality, increases their sugar content and decreases their acidity (Table 6).

However, while the foliar feeding of cherry with NPK + B in the concentration we selected leads to an increase in fruit size, the opposite phenomenon is observed in apple. The fruit of the trees was studied only in 1957, which of course is as yet not sufficient for a definite conclusion. It is possible that the decrease in fruit size in apple in 1957 may be explained by the insufficiency of root feeding while the number of apples initiated as a result of spraying is larger.

SUMMARY

1. Foliar feeding of apple and cherry with a mixture of boron increased the activity of a number of oxidation-reduction enzymes in leaves: dehydrases, catalase, peroxidase, ascorbic acid oxidase, while polyphenol oxidase activity decreased in a majority of the varieties studied.

2. After spraying with nutrient substances, the content of ascorbic acid, iodine-reducing substances, and general oxidizability of the cell content in leaves are increased. The pH of the cell content did not vary appreciably during aerial feeding.

3. Because of the general activation of physiological-biochemical processes in leaves, foliar feeding considerably increases yield, decreases the fall of ovaries, and improves the taste qualities of the fruit obtained.

4. As a result of observations over three years, one may conclude that foliar feedings are effective for fruit crops under the conditions of Saratovskaya region.

LITERATURE CITED

1. S. S. Rubin, Fertilization of Fruit and Berry Crops [in Russian] (Izd. AN SSSR,1949).
2. P. K. Ursulenko Coll.: Foliar Feeding of Agricultural Crops [in Russian] (Sel'khozgiz, 1955).
3. Ya. A. Mednis, Kolkhoz Proizvodstvo, No. 6 (1950).
4. T. N. Godnev, N. S. Sudnik, and É. N. Syubarova, Uch. Zap. BGU, No. 26 (1956).
5. I. I. Gunar and M. I. Kalinkevich, Moskovskii Kolkhoznik, No. 5 (1956).
6. D. Boynton and E. G. Fisher, Proc. N. Y. State Hort. Soc. 94, 101 (1944).
7. D. Boynton, E. Fisher, and K. Skodvin, Proc. Am. Soc. Hortic. Sci 51, 23 (1948).
8. C. H. Blasberg, Foliar Feeding of Plants. Coll. of Translations from the Foreign Periodical Literature, edited by Shkonde (1956).
9. P. A. Vlasyuk and Z. M. Klimovitskaya, Nauchnie Tr. IFR i Agronomii AN UkrSSR No. 3 (1951).
10. S. M. Mashtakov, Izvest. AN BSSR, No. 6 (1952).
11. Ya. V. Peive and A. E. Krauya, Doklady Akad Nauk SSSR 117, No. 5 (1957).*
12. V. V. Yakovleva, Foliar Feeding of Agricultural Plants, Collection [in Russian] (1955).
13. B. A. Rubin and N. M. Sisakyan, Biokhimia 4 (1939).
14. B. A. Rubin and N. N. Sisakyan, Doklady Akad Nauk SSSR 25, No. 4 (1939).
15. A. A. Shakhov, Salt Resistance of Plants [in Russian] (Izd. AN SSSR, 1956).
16. B. A. Rubin and E. V. Artsikhovskaya, Biokhimia 2 (1937).
17. B. A. Rubin and O. T. Lutikova, Doklady Akad Nauk SSSR 17, No. 5 (1937).
18. S. Thurlaw, Biochem. J. 19, 175 (1925).

* See English translation.

TRANSFORMATION OF RESERVE SUBSTANCE IN FLOWER BUDS OF BLACK CURRANT DURING THE ANNUAL CYCLE

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A number of investigations [1-4, etc.] have been carried out to determine the transformation of reserve nutrient substances in woody and shrub plants; these indicated that the degree of transformation of nutrient reserve substances together with the change in properties of protoplasm are criteria for determination of the depth of dormancy and frost resistance of plants.

In woody and shrub plants, transformation of substances during passage into the dormant state takes place up to formation of lipoids which are accumulated on the surface of protoplasm, which decreases the excretion of water from the cell and prevents its penetration from outside. These changes lead to the insulation of protoplasm and contribute to its resistance to the action of ice crystals, which are formed in intracellular spaces.

The deeper the transformation of substances, for example, the formation of fats and protein-lipoid compounds, the greater is the winter and frost resistance. The more secure the linkage of lipoids and tannin-lipoid compounds with protoplasmic proteins, the more resistant the cells are to the action of unfavorable factors.

In a number of nondeciduous plants of various types, Lidforss [5] had observed the complete absence of starch in their leaves and an accumulation of sugars during the winter. He studied microchemically 130 plants belonging to 40 different families, and in all he found clearly defined winter saccharophily. On the basis of his observations, Lidforss concluded that sugars have a protective action against frost. He also points out that sugar accumulation is only one of the factors in frost resistance; a number of other very important factors are present along with this.

The same conclusions concerning the reasons for frost resistance were later derived by N. A. Maksimov, who worked on respiration of plants at temperatures below 0° [6].

Protsenko and Polishchuk [7], working with fruit crops, have established that the more frost resistant plants are characterized by an increased sugar content. According to their data, the total number of monosaccharides in fruit crops is higher in the winter than it is in the fall. Disaccharides have been found to be more plentiful in the fall than in the winter. Oknina and Pustovoitova [8] have noted the presence of glucose and fructose in the flower buds of apple and cherry, while in cherry buds they have also found a sugar from the pentose group. According to their observations, the content of soluble sugars is sharply decreased when buds come out of dormancy. Sucrose and oligosaccharides have not been found in the buds in the winter by these authors. The absence of sucrose in buds during this period may be connected with the very small content of free amino acids at this time, and with absent or very slow translocation of substances of all tissues with insulated protoplasm.

The position of fats, proteins, lipoids, tannin substances, and complexes from tannin-lipoid and protein-lipoid compounds as reserves makes possible prolonged existence of plants with decreased metabolism in the tissues and organs, and contributes to the conservation of the maximum amount of reserve nutrient substances, which are necessary for the normal spring growth [6,8]. Poyarkova [9] has established that the transformation of substances in the shoots of black currant does not proceed as far as the formation of the fats and that reserve substances are deposited as starch and soluble carbohydrates, which does not agree with our observations, reported in this paper.

The present paper is a continuation of our study on the dormant state in fruit plants, in particular black currant, in relation to winter resistance. We have followed the dynamics of reserve substances in the annual cycle of flower buds in black currant from the moment of their initiation in the summer through the dormant state, budding and flowering, to the formation of fruits.

The transformation of substances in the flower buds of black currant has been studied in longitudinal sections through the buds, using the microchemical methods proposed by Genkel' and Oknina [10]; the content of soluble sugars and of free amino acids was studied by the method of chromatography, developed by Boyarkin [11]. Calculation of the amount of these substances and starch was carried out to 5 significant figures.

As experimental objects the first year's shoots, flower buds, flowers and fruits of black currant, variety

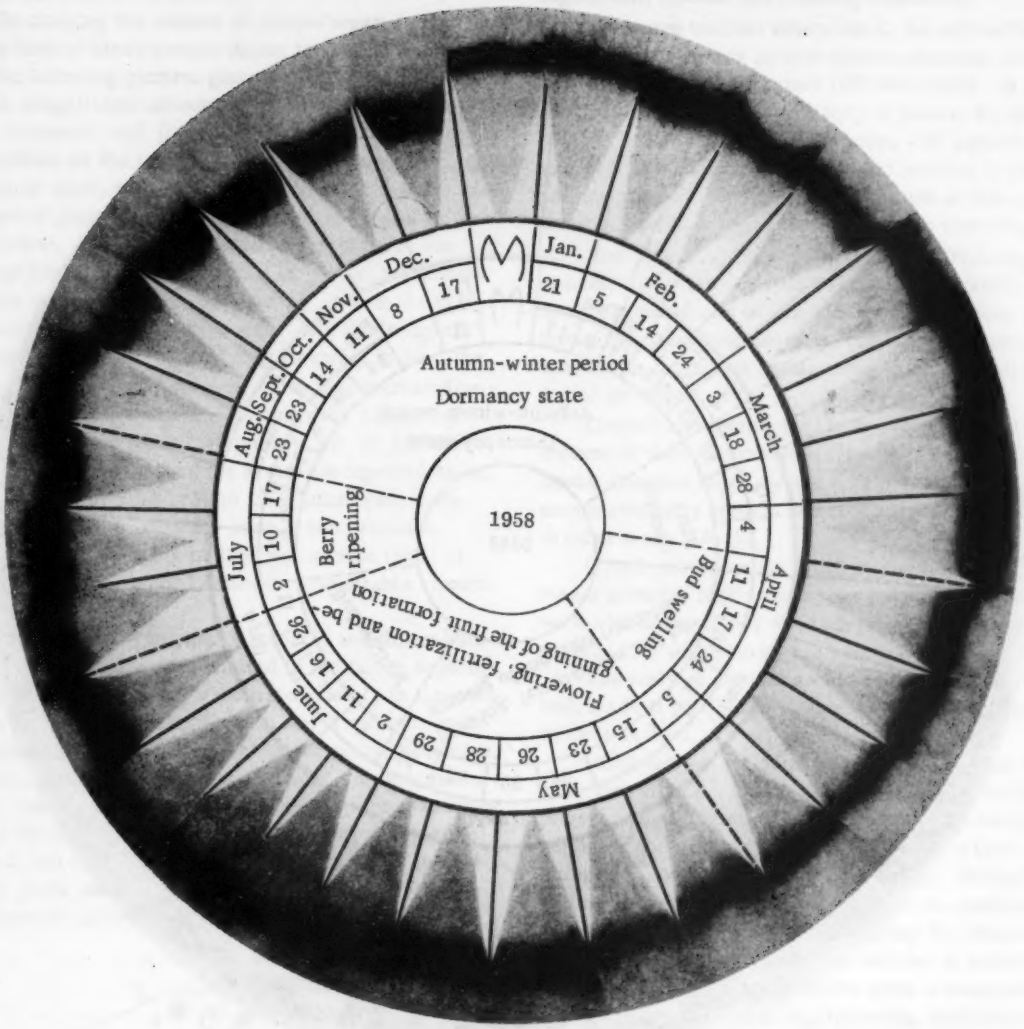
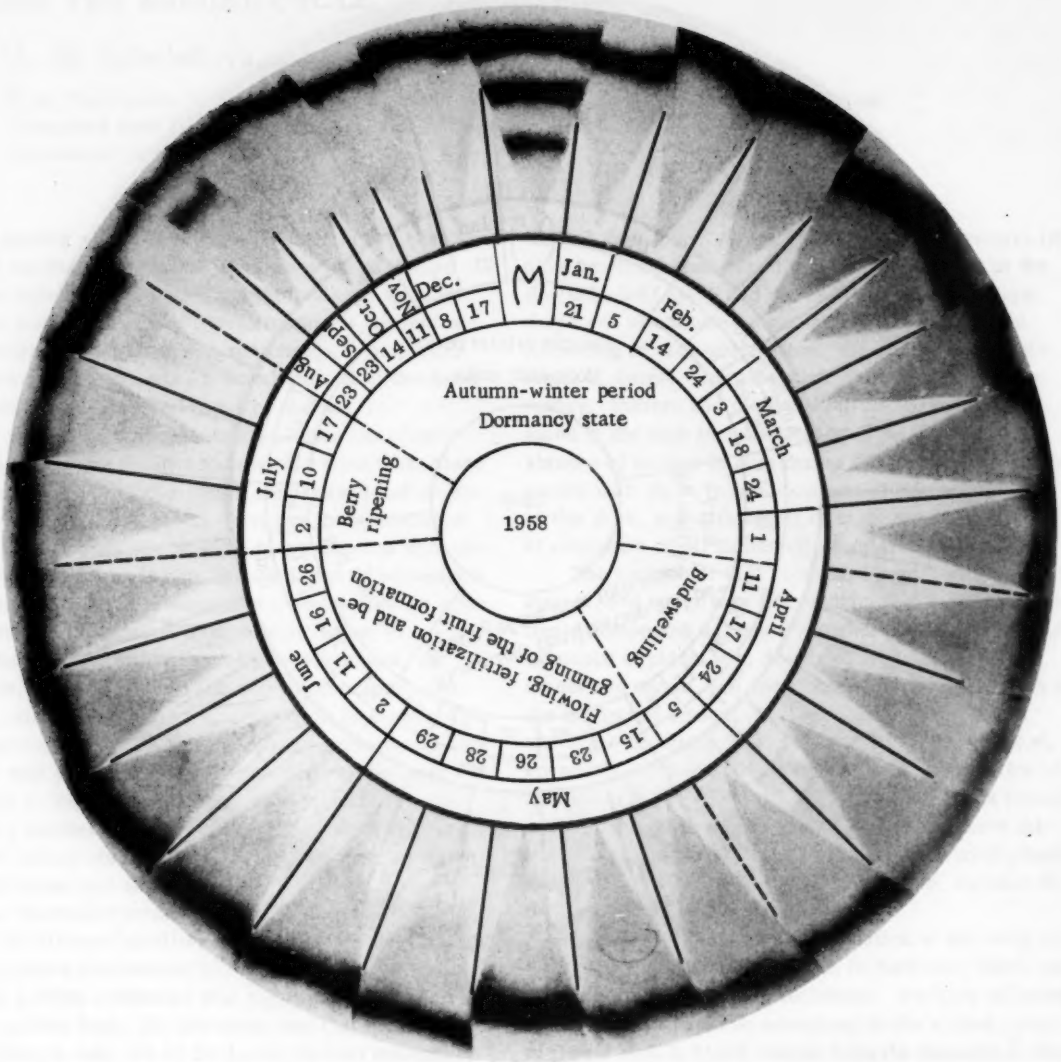


Fig. 1 A

Fig. 1. Content of glucose (A) and fructose (B) in flower buds of black currant.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Autumn-winter period
Dormancy state
1958
Berry ripening
Budswelling
Flowing, fertilization and be-
ginning of the fruit formation

Fig. 1 B

Lia plodorodnaia, were used. Samples were taken from the experimental plot of the Institute of Plant Physiology, Academy of Sciences of the USSR, located on Lenin's Hills, once a month during winter, and three to four times in the spring and summer. The work has been carried out from 1956 to 1959.

On studying the content of soluble sugars in the flower buds of black currant during the annual cycle, we saw the following picture: glucose and fructose were present in insignificant amounts in September; their contents then increased and from October until April were held almost on the same level (up to 4 points); from the middle of April, at the time of opening of the buds, the amount of glucose and fructose decreased (down to 1-2 points). Then in the second half of May, at the time of flowering and emergence of leaves, the amount of these sugars again was considerably increased (up to 3 points); in June again there were less soluble sugars (2 points), but at the time of fruit ripening, in July, the amount of glucose and fructose was sharply increased (up to 4 points). In August and September the amount of soluble sugars was small (1-2 points), but in October their amount increased and the cycle was repeated anew; we observed this from 1956 to 1959, aside from variations due to variation in the average temperatures. Only once, in September of 1958, was sucrose found in the flower buds of black currant in considerable amount (3 points) (Fig. 1).

The maximum amount of starch in the flower buds of black currant was accumulated in the fall in September and October; it was concentrated mainly in the cells of the receptacles. A decrease in starch content, to its complete disappearance, was observed during December, January, February, and March. In April, an increase in starch content was noted, the so-called spring maximum, but in distinction from the fall maximum, at this time there is less starch in the cells than in the fall, and the starch grains are smaller. The spring maximum lasts a considerably shorter time than the fall maximum. On the average, the starch content during the spring maximum may be evaluated as 2.5-3.0 points as distinguished from the fall maximum, when its amount is evaluated as 4 points. The decrease in starch content during winter months is connected with its transformation into sugars, fats, lipoids, and reducing substances.

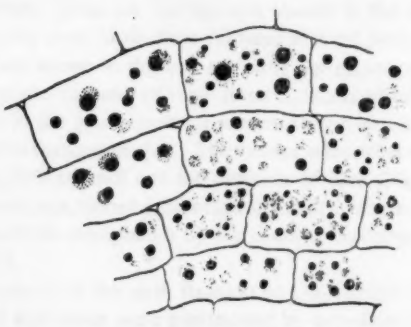

Fig. 2. Breakdown of protein-lipoid substances and starch formation in flow buds of black currant. Fixed March 27, 1958. Beginning of breakdown of dormancy in the tissues of the buds.

As reserve nutrient substances in the cells of black currant buds, complex protein-lipoid substances are also deposited, which are retained [12] until April. In April, at the time of breaking dormancy in tissues, the breakdown of these compounds takes place with formation of starch; the separation of lipoids from proteins is observed and the appearance of starch can be seen at first as very small grains, which gradually increase in size (Fig. 2).

The data on the change in content of reducing substances, which are determined by means of osmic acid, in annual shoots and flower buds of black currant have shown that these substances are present in considerable amounts in the tissues during fall and winter until breaking of dormancy.

Genkel' and Oknina [10] have noted that a greater content of these substances is observed in the tissues of winter-resistant plants, which contain more fats with unsaturated fatty acids. This conclusion was confirmed in other works [13-15].

Lipoid substances are found in the protoplasm by means of Sudan black reagent, as a darkly stained network with nodes and by Sharlach stain as a pinkish-red staining also with pinkish-red nodes.

The study of the free amino acid content in flower buds made it possible to establish the seasonal variations in their amount.

In August, two amino acids were found in the flower buds; then from September until April, a few acids are present, mainly proline. Proline is present during all periods of the year, in great amount in the winter and the spring, and only in traces during June. Evidently, proline is one of the amino acids which is accumulated as a reserve. In April, May, and during the breaking of dormancy in the flower buds, the number of amino acids rises to 8. This number of amino acids is maintained during the periods of budding, flowering, fertilization, and fruit bearing. In the newly initiated buds (at the end of July), the free amino acid content is very small.

In samples taken in February, March, April, and May of 1958, in addition to proline, certain unidentified substances were found, developed by isatin on the chromatogram as yellow and brown stripes between methionine and valine and between leucine and phenylalanine. The nature of these substances is not clear. Histidine is shown as traces in November, December, and January; in other months it is not found. Arginine is found in noticeable amounts in the fall, but from February till April it is present only as a trace. Large amounts of arginine are found in the buds in June. Aspartic acid is found as traces in June and July. Glutamic acid is

absent in the fall and is found in considerable amounts in February, and then it is kept on a low level in June and July.

Threonine is found in buds in small amounts during the winter, but in April and May, during bud opening and flowering, its amount increases. Valine and leucine were found only during the period of berry ripening.

From this data it may be concluded that the amides of asparagine and glutamine, aspartic acid, valine and leucine are acids which are synthesized by plants during their active growth and development: opening of the buds, flowering, and ripening of the fruit; during the period of dormancy of flower buds these acids are not found, as a rule. Thus, the increase of a number of free amino acids takes place from the moment of breaking out of dormancy in black currant, and reestablishment of protoplasmic connections.

These data concerning the large number of free amino acids in buds which have emerged from the state of dormancy once more support the conclusion of Genkel' and Oknina [10,18] concerning the fact that during dormancy, when the protoplasm in the cells is insulated, a decrease in metabolic rate takes place, while from the moment of dormancy breakdown and reestablishment of protoplasmic connections, the metabolic rate increases, and the breakdown of protein-lipoid compounds takes place, with the formation of free amino acids, sugars, and starch.

SUMMARY

A deep relationship was established between tannin-lipoid substances and proteins of protoplasm, and there is a prolonged stable state of complex protein-lipoid substances. During their breakdown, at the time of breaking dormancy in the tissues, in April, a simultaneous formation of starch is observed. A deep transformation of reserve nutrient substances in the state of dormancy, in black currant, characterizes the plant as a frost-resistant plant.

LITERATURE CITED

1. A. S. Grebnitskii, Tr. St. Peterb. Obshchestva Estestvoispyt. XV, No. 2 (1884).
2. E. P. Votchal, Tr. Kazansk. Obschestva Estestvoispyt. pri Univ., 104 (1888).
3. I. I. Tumanov, Main Achievemnts of Soviet Science in the Study of Frost Resistance of Plants [in Russian] XI TimiryazevskoeChtenie. (Izd. AN SSSR, 1951).
4. I. I. Tumanov, Fiziol. Rastenii 2, 283 (1955).
5. E. Lidforss, Die wintergrüne Flora. Lunds Universitets Arsskrift N. F. 2, Afd. 2, No. 13.
6. N. A. Maksimov, Selected Works on Drought Resistance and Winter Resistance. Winter Resistence of Plants [in Russian] (Izd. AN SSSR, 1952), Vol. II.
7. D. F. Protsenko and L. K. Polishchuk, Physiological and Biochemical Characteristics of Frost Resistance in Fruit Crops [in Russian] (Izd. Kiev. Univ. im. T. G. Shevchenko, 1948).
8. E. Z. Oknina and T. N. Pustovoitova, Summaries of Reports in the Conference on Physiology of Plants Resistance [in Russian] (Izd. AN SSSR, 1959).
9. A. I. Poyarkova, Tr. Leningr. Obshchestva Estestvoispyt. 54, No. 3 (1924).
10. P. A. Genkel' and E. Z. Oknina, Study of the Dormancy Depth in Woody Species for the Diagnosis of their Frost Resistance [in Russian] (Izd. AN SSSR, 1952).
11. A. N. Boyarkin, Fiziol. Rastenii 2, 299 (1955).
12. E. Z. Oknina and E. I. Barskaya, Izvest. AN SSSR, Ser. Biol., 5, 104 (1954).
13. T. P. Petrovskaya, Byul. Gl. Botan. Sada AN SSSR, 13, 72 (1954).
14. E. Z. Oknina and E. I. Barskaya, Coll.: Essays in Memoriam Academician N. A. Maksimov [in Russian] (Izd. AN SSSR, 1951).
15. P. A. Genkel' and E. Z. Oknina, Tr. Inst. Fiziol. Rast. AN SSSR VI, No. 1 (1948).

BRIEF COMMUNICATIONS

EFFECT OF EDAPHIC DROUGHT ON DARK FIXATION OF CO_2 BY WHEAT LEAVES

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It is known that during dark fixation of $C^{14}O_2$, the radiocarbon is detected principally in the organic acids of the Krebs cycle and genetically coupled amino acids [1,2]. The effects of environmental conditions on chemical aspects of the dark fixation of gaseous carbon dioxide have been studied very little. Only in the laboratory of Nichiporovich has work been conducted pertinent to elucidating the effect that various nitrogen regimes have on the dark fixation of CO_2 by plants [3].

We set ourself the task of discovering how soil drought affects the formation of the products of dark fixation of CO_2. Hard winter wheat, variety Gordeiforme 496, during the tillering stage was used. The plants were raised in greenhouse pots in forest podzol soil fertilized with $N_{45}P_{60}K_{60}$. We studied wheat leaves which developed under conditions of optimal water supply (soil moisture 65% of field capacity) and with soil drought (the sample was taken July 18, 1958, when the leaves of the upper storeys began to wilt. The upper storey leaves were cut off and placed in a ventilated light cabinet through which $C^{14}O_2$ was passed (1.5 volume percent). The leaf samples weighed about 3 g; the period of dark fixation was 25 min. Each treatment was replicated four times. After the leaves were placed in the lighted cabinet no more than three minutes elapsed before the $C^{14}O_2$ was blown in, thereby avoiding the effect on the "path of the carbon" of long-lived reducers which are formed in the photosynthesis process in the opinion of certain investigators. [4]. The leaves were treated with boiling 80% ethanol and the products of dark CO_2 fixation were partitioned into ethanol-soluble, water-soluble, and insoluble compounds by the method described by one of us [5].

Products of the dark fixation of CO_2 soluble in ethanol and water were partitioned by two-directional paper chromatography using butanol: formic acid: water (75:13:12) and water-saturated phenol. The chromatograms were laid on an x-ray film and exposed one month. After the radioautographs were obtained, the compounds were eluted from the chromatogram and their radioactivity determined with an end-window counter. During the determination of radioactivity in the insoluble residue, a correction was made for self-absorption.

For determination of the water regime of the leaves studied, samples were taken for total water content (5 replicates), free water (4 replicates) and osmotic pressure (3 replicates). Free water and osmotic pressure were determined with a refractometer. The free water was defined as that extracted from the leaves by a 30% sucrose solution in two hours. Curtailment of irrigation caused a change in the water regime of the upper storey leaves of wheat (Table 1).

The total water content and the amount of free water decreased and the osmotic pressure increased during edaphic drought. This might affect the chemistry of dark fixation of CO_2.

From the radioautographs it has been found that a number of the products of nonphotosynthetic fixation of carbon dioxide gas are the same in wheat leaves grown both under conditions of optimal water supply and in edaphic drought. Radioactive carbon is detected in six compounds: malic, aspartic, glutamic, and fumaric acids, and alanine and serine. A trace of chlorophyll appeared on the radioautographs but it seems that even nonlabeled chlorophyll leaves a black spot on the x-ray film. The radioactivity of the malic, aspartic, and glutamic acids was determined after extraction from the chromatogram. The radioactivity of the fumaric acid, alanine and serine was not determined since it was very low. It appeared that edaphic drought caused an appreciable change in the rate and direction of dark fixation of CO_2 (Table 2).

From the data of Table 2 it appears that edaphic drought reduces to one-half the rate of dark fixation of CO_2 by wheat leaves. This is perhaps explained by either a reduction in the rate of penetration of CO_2 gas into the cells or a decrease in available CO_2 acceptors.

During edaphic drought the amount of radiocarbon decreases in the insoluble fraction (protein, polysaccharides and lipids insoluble in 80% ethanol). The latter, in all probability, is a result of the long-established fact

TABLE 1. Effect of Edaphic Drought on Amount of Total and Free Water (% dry wt) and Osmotic Pressure (in atm) in Leaves of Wheat

Treatment	Total water	Free water	Osmotic pressure, atm
Optional water supply	76.5±0.4	28.2±0.9	8.2±0.1
Edaphic drought	65.2±2.6	13.5±1.2	14.7±0.2

TABLE 2. Effect of Edaphic Drought on Rate and Direction of Dark Fixation of CO_2

Radioactivity	Optional water supply	Edaphic drought
Total c/m x 10^{-3}	1504±67	711±53
Insoluble compounds (% of total)	2.1±0.2	1.2±0.1
Soluble compounds (% of total)	97.9±0.2	98.8±0.1
Aspartic acid*	69.4±2.3	54.5±0.1
Glutamic acid*	10.8±1.8	10.2±0.1
Malic acid*	19.8±1.4	35.3±1.2

* % of radioactivity of soluble compounds.

that the formation of compounds of high molecular weight from compounds of low molecular weight is inhibited during drought [6]. The relative amount of C^{14} increases in the soluble fraction of wheat leaves grown under soil drought. This shift in direction of the chemistry of dark fixation of CO_2 toward a more rapid formation of compounds of low molecular weight is just one evidence of a general tendency: an increase in the amount of low molecular weight, osmotically active compounds in plant tissues during drought. Of course the changes in specific gravity during dark fixation of CO_2 and the increase in osmotic pressure of the cell sap are not great.

Edaphic drought caused a change in the proportion of products of CO_2 dark fixation and within the soluble fractions: an increase in the radioactivity in malic acid and a decrease in aspartic acid. The formation of aspartic acid during the dark fixation of CO_2 can be represented as follows. The nonphotosynthetic acceptor of CO_2 is, apparently, pyruvic acid (or phosphoenol-pyruvate), which is readily carboxylated to form oxalic acid. The latter is rapidly converted to malic acid. The malic acid is converted to aspartic by amination. Proceeding from this hypothesis we can explain the increase in C^{14} content of malic acid and a decrease in aspartic during drought as a decrease in the speed of the "nonphotosynthetic carbon pathway" and a reduction in the rate of conversion of malic to aspartic acid. We have described earlier [7] the slowdown in the speed of the "carbon photosynthetic pathway" in the plant during edaphic drought conditions.

LITERATURE CITED

1. L. A. Nezgovorova, Doklady Akad. Nauk SSSR 86, 4, 853 (1952).
2. L. Poel, J. Exp. Bot. 4, 157 (1953).
3. L. A. Nezgovorova, Fiziol. Rastenii 3, 343 (1956).
4. M. Calvin, J. A. Bassham, and A. A. Benson, Federat. Proc. 9, 2 (1950).
5. I. A. Tarchevskii, Bot. Zhur. 4, 513 (1959).
6. N. M. Sisakyan, Biochemical Characteristics of Plant Drought Resistance [in Russian] (Izd. AN SSSR, 1940).
7. I. A. Tarchevskii, Trudy Kazanskogo Sel. - Khoz. Inst., No. 36 (1957).

CERTAIN PECULIARITIES OF WHEAT GRAINS INFESTED WITH THE CHINCH BUG

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The chinch bug (Eurygaster integriceps Put.) infesting the wheat grain locally, in a majority of cases [1-3], apparently disturbs the usual pattern of physiological processes of the tissue infested. These changes may also lead to an inhibition of growth and development, and finally, to a reduction of productivity of plants which develop from the infested grain.

The investigations of Blagoveshchenskii [4, 5], Kretovich, et al. [1,2,3,6], Proskuryakov and Bukharina [7], and others, have been devoted to study of the biochemical characteristics of grains of wheat infested by the chinch bug. The physiological features, however, have been investigated very little.

We studied the viability of wheat grains with tissues damaged by the chinch bug by observing their ability to germinate. Grains infested in the embryo zone did not germinate. During the process of germination, fungi, predominantly Alternaria, develop abundantly in the embryo tissues of these infested seeds. The germination of grains infested outside the embryo appears poorer than that of healthy grains. The greatest lag in germination of infested grains was observed during the first three days of germination (Table 1). The seedlings of infested grains developed appreciably more weakly than seedlings of healthy grains. A decrease in dry weight of infested seedlings is apparently explained by a slower utilization of the materials stored in the endosperm of infested seedlings.

We also investigated the viability of infested grains by studying the staining of the grain (in section) with a 0.1% aqueous solution of indigo-carmine after the seeds were hot-water treated.

Using this method and luminescent microscopy of sections of the grain we were able to observe the appearance of necrosis in infested tissues of the grain. We observed a similar appearance in samples of the grain after six months or a year of storage.

We investigated in the following way the respiration rate in grains infested with chinch bugs. We determined the respiration rates of whole grains infested with chinch bugs.

TABLE 1. Germination and Weight of Seedlings From Wheat Grains Infested with Chinch Bugs

Wheat variety and region	Germination, %			Dry weight of seedlings (mg/grain)		
	healthy	infested	healthy: infested	healthy	infested	healthy: infested
Odessa 3*						
Baltiiskii	40	40	1:1.00	27.0	20.0	1:0.74
Sinel'nikovskii	96	46	1:0.48	32.0	25.0	1:0.78
Belozerskii	52	44	1:0.85	25.0	23.0	1:0.92
Odessa 16*						
Baltiiskii	48	42	1:0.88	36.0	17.0	1:0.47
Sinel'nikovskii	90	64	1:0.71	30.0	25.6	1:0.85
Belozerskii	90	70	1:0.78	24.4	20.0	1:0.82
Odessa 16**						
Odessa genetic selection institute	63	33	1:0.52	47.2	15.7	1:0.33
	98	87	1:0.89	107.0	64.3	1:0.60

* Grain harvest 1957, germinated in 3 days

** Grain harvest 1958, data on the lower line relate to grains which germinated in seven days, those on the top line in three days.

TABLE 2. Respiration of Grains Infested by Chinch Bugs ($MgCO_2$/hr · g dry wt)

Wheat variety and region	Infection %	Harvest, 1956			Harvest, 1957		
		healthy	infested	respiration healthy: infested	healthy	infested	respiration healthy: infested
Odessa 3							
Baltiiskii	2.5	0.54	0.57	1 : 1.05	0.49	0.57	1 : 1.16
Sinel'nikovskii	0.8	0.62	0.68	1 : 1.09	0.48	0.55	1 : 1.15
Belozerskii	0.3	0.58	0.65	1 : 1.12	0.48	0.51	1 : 1.01
Zhmelevskii	4.5	0.38	0.54	1 : 1.42	0.52	0.63	1 : 1.26
Odessa 16							
Baltiiskii	2.5	0.65	0.71	1 : 1.09	0.38	0.46	1 : 1.21
Sinel'nikovskii	0.7	0.92	0.96	1 : 1.04	0.58	0.69	1 : 1.20
Belozerskii	1.1	0.72	0.74	1 : 1.03	0.69	0.74	1 : 1.07

TABLE 3. Respiration of Odessa 16 Wheat Grains Infested with Chinch Bugs and Infested Tissues (MgO_2 absorbed/hr·g dry wt)

Date	Harvest year	Grain			Tissue					
		healthy	infested	healthy: infested	healthy	infested	healthy: infested	adjoining infested	nonadjoining	nonadjoining: adjoining infested
Mar. 30, 1959	1956	15.0	16.3	1 : 1.1	21.4	72.8	1 : 3.4	38.1	27.9	1 : 1.4
Mar. 30, 1959	1957	26.4	31.3	1 : 1.2	23.4	69.1	1 : 2.9	—	—	—
Dec. 13, 1958	1958	9.3	68.0	1 : 7.3	29.5	98.4	1 : 3.3	—	—	—
Mar. 30, 1959	1958	11.7	16.0	1 : 1.4	21.0	49.3	1 : 2.3	42.0	19.9	1 : 2.1

of individual infested tissues separated from the grain, and of healthy tissues of the same grain. We set up the experiments considering the time factor by using the samples of grains of certain harvest years and periodically conducting tests with the same seeds. Respiration rate was determined on the basis of the volume of CO_2 evolved [8] and manometrically by the amount of oxygen absorbed. Grain moisture was 11.5-12%.

The experimental results showed that the respiration of infested grain exceeded that of the healthy controls. The respiration rate of infested grain decreased with time as evidenced by the samples from the 1956 and 1957 harvests (Table 2).

A series of experiments definitely showed that the respiration rate of wheat grains increases when infestation by chinch bugs is present. Tissue of grains infested by chinch bugs respired 2.5 times more rapidly than did the healthy wheat grains. The initial sharp increase in respiration of infested grains from the 1958 harvest diminished during the subsequent determinations (Table 3). In the majority of cases, the rate of respiration of infested grains increased within a variety as the degree of damage increased.

It is recognized that the rate of respiration is greater in diseased than healthy tissues [9,10] and the affected tissue typically has greater enzyme activity, particularly the activity of oxidative enzymes.

The nature of the respiratory metabolism requires that there be present: 1) Secretions of the bug including a number of active enzymes; 2) grain enzymes freed through tissue destruction; 3) products of grain destruction and, perhaps, 4) toxic substances. The latter may be formed as a result of the death of tissues and move into neighboring tissues where they perform a protective function similar to a low dose of a substance which increases the tone of the living processes and in the presence of which are created optimum conditions for resisting and surviving the infestation [9].

Investigation of the respiratory rate of tissues bordering the infested tissue of the grain attest to the high physiological activity of this tissue (Table 3).

Interruption of the oxidation-reduction processes of infested grain, apparently, is never reflected in the features of these processes in plants developing from the given grain.

We investigated the respiration of four-day-old coleoptiles of healthy and infested grains of two varieties of wheat (Odessa 16 and Odessa 3) infested by chinch bugs. The results appear as follows:

Wheat coleoptile	Absorption (ml/hr · g wt)	Respiration rate healthy: infested
Odessa 16 grain:		
Healthy	275.1	1 : 1.37
Infested	378.3	
Odessa 3 grain:		
Healthy	315.8	1 : 1.19
Infested	376.6	

From the experimental data that we have obtained it is apparent that infestation of wheat grains with chinch bugs deranges the vital functions of the grain which may lead to a reduction in plant efficiency and productivity.

LITERATURE CITED

1. V. L. Kretovich and R. Tokareva, Biokhimiya 3, 387 (1938); 4, 636 (1939).
2. V. L. Kretovich, K. Pshenova, and A. Bundel', Doklady Akad. Nauk SSSR 40, 1, 35 (1943).
3. V. L. Kretovich, Biochemistry of Grains and Bread [in Russian] (Izd. AN SSSR, 1958).
4. A. V. Blagoveshchenskii and N. I. Sosedov, Biochemistry and Microbiology of Wheat [in Russian] (Izd. VNITZ, 1934) No. 13.
5. A. V. Blagoveshchenskii, Biochemistry of Metabolism of Nitrogenous Materials in Plants [in Russian] (Izd. AN SSSR, 1958).
6. V. L. Kretovich, K. Pshenova, and A. Bundel' Doklady Akad. Nauk SSSR 39, 1, 31 (1943).
7. I. I. Proskuryakov and E. V. Bukharina, Biochemistry of Baked Bread [in Russian] (Izd. AN SSSR, 1942) 3rd ed.
8. E. S. Turlygina, Doklady Akad. Nauk SSSR 115, 6, 1227 (1957).
9. V. F. Kuprevich, The Physiology of Diseased Plants [in Russian] (Izd. AN SSSR, 1947).
10. K. T. Sukhorukov and P. V. Cherepanova, Byul. Glav. Botan. Sada. AN SSSR, No. 14 (1952).

RELATION BETWEEN THE WATER CONDITIONS IN FRUITS AND LEAVES

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Shriveling, cracking, and premature fruit drop are undesirable phenomena observed in the orchards of the central Asiatic Republics. All of these result in a decrease of yield and quality and constitute a considerable material loss to the fruit producing economy. Cracking of pomegranate fruits in southwest Turkmen causes an especially great loss. The fundamental cause for this cracking has not been studied and thus methods necessary to eliminate it have not been devised [1,2].

At the experimental station of the All-Union Arid Subtropics Scientific-Research Institute our three years of observation on the behavior of fruits and leaves of different varieties of pomegranate showed that the leaves on detached branches without fruits dried up in 15-18 hours, while leaves on branches bearing fruits retained their crispness up to 72 hours or more. The more leaves there were per fruit and the smaller the diameter of the fruit, the more rapidly it began to shrivel. Analogous effects were observed with peach leaves and fruits. These observations serve as a basis for the hypothesis that the leaves extract water from the fruits during periods of plant water deficit. Leaf extraction of water from the fruits, particularly during abrupt fluctuations in plant water balance, causes cell damage in the fruits and a separate layer is formed at the place where the fruit stem is attached to the fruit receptacle, leading to the phenomena mentioned. This suggestion is also based on such indicators as transpiration, suction force and osmotic pressure of the cell sap.

It is known that transpiration, suction force, and osmotic pressure of the cell sap are greater in the leaves than in the fruit and other plant parts. The leaves, in predomination over the other parts of the plants, extract water from the fruits during a period of plant water deficit.

We conducted suitable experiments and obtained corroborative data in order to verify the correctness of this hypothesis. The experiments were carried out in the laboratory of plant physiology of the All-Union Arid Subtropic Scientific Research Institute (VNIISS). The experimental objective was to reveal the loss of water by various parts of plants both separately and in combination.

The following experimental methods were used to solve this problem. At the VNIISS experimental station similar branches bearing equal numbers of leaves and fruits were cut from four varieties of pomegranate (Kzyl, Kazake, Kadan, Baisunskii) and three varieties of peaches (Elberta, Khedistanskii, and Sol'vei). Immediately after they were cut, the branches were placed in vessels of water in the laboratory. All leaves and fruits were stripped from one branch, from another, only the fruits, from a third, only the leaves, and the fourth was left unaltered. Thus, when the experiment began, we had leaves, branches, and fruits separately, and leaves on branches along with fruits. Such a dissection permitted us to adjudge the loss of water from each plant part both separately and in relation to other parts at the same time. Each dismembered part was weighed initially and after 6, 12, 18, and 24 hours had elapsed. The difference between the initial and subsequent weights permitted a judgement of the water in each plant part at the same time. Water loss from leaves on branches without fruits and from leaves on branches bearing fruits was determined somewhat differently. A part of the leaves was removed from the branches initially, and subsequent parts after 6, 12, 18, and 24 hours. Then each portion of the leaves removed was weighed, submerged in water for 5 hours, and reweighed. Through the difference between the weights of water-saturated and unsaturated leaves we can judge the water deficit of cut off branches both as to the water loss of leaves on branches without fruits and on branches bearing fruits during successive periods.

Leaf water saturation prior to the initial condition was determined as follows. Leaves simultaneously stripped from the branches were divided into five equal portions, which were then weighed. The first of these portions was immediately submerged in water, the second after 6 hours, and then the rest after 12, 18, and 24 hours had elapsed. Each portion of leaves was weighed prior to submergence in water and after 5 hours in water. There was an exceedingly small difference obtained be-

Water Loss by Leaves of Several Varieties of Pomegranate and Peach at Various Times After Being Cut Off (in percent of initial weight)

Variety	Leaves on branches without fruit				Leaves on branches having fruits			
	6 hr	12 hr	18 hr	24 hr	6 hr	12 hr	18 hr	24 hr
Pomegranate								
Kzyl	32	42	55	55	14	24	26	31
Kazake	17	28	46	52	4	10	18	22
Kadon	26	41	52	58	7	11	17	23
Baisunskii	16	29	41	57	7	8	13	15
Peach								
Elberta	34	45	50	52	20	24	29	35
Khedistavskii	22	30	40	46	14	15	16	19
Sol'vei	17	29	34	41	7	12	14	22

tween the initial leaf weight and the weight after saturation with water. It was less during the morning and more during the midday hours.

The temperature of the air where the experiment was conducted fluctuated in August from 35° during the day to 30° at night and during September, from 24° during the day to 21° at night.

The experiments conducted show a definite relation between the water regime of different parts of the pomegranate and the peach. The leaves lose the most water, the branches less, and the fruits still less. The twenty-four hour water loss by separate plant parts is: up to 62% by pomegranate and peach leaves, pomegranate branches up to 29% and peach branches up to 37%, pomegranate fruits 7% and peaches 12% of the initial weight. The same parts of the plant in the different varieties lose different amounts of water. Leaves on branches without fruits lost appreciably more water than branches having fruits, as can be seen from the table.

Here it should be noted that fruits on branches bearing leaves shrivel appreciably earlier than they do on branches without leaves, indicating the ability of the leaves to extract water from the fruits during a period of plant water deficit. This supports the correctness of our hypothesis concerning the reason for fruit damage and premature drop. No doubt, only during favorable conditions of plant water supply are the leaves able to supply the fruits with necessary materials for their normal growth and development. On the other hand, during plant water stress the leaves can extract water from the fruits so long as the plant water balance is not restored or until the stored water in the fruit is used up. In brief, during conditions favorable for photosynthesis the leaves nurture the fruits, but during unfavorable conditions water is extracted from the fruits. During periods of plant water deficit the fruit is a reservoir from which the leaves draw water. Water loss from the fruits leads to shriveling, damage to cells and tissues, splitting and cracking, the formation of an abscission layer in the fruit petiole, and premature fruit drop, which are linked with a decreased fruit yield and quality.

From this it follows that difficulties with increasing fruit yield and quality must be closely connected with difficulties in normal plant water supply during the entire vegetative period. Deficiency or excess of soil moisture are, similarly, injurious both to plant growth and development and to fruit quantity and quality. Plant water supply must conform to the biological peculiarities of the plant and its water requirement. In this regard, it is necessary to consider that the drier the air, the higher the temperature, the stronger the insolation, and the greater the plant leaf surface the more copious the soil moisture must be.

LITERATURE CITED

1. N. A. Maksimov, Trudy Inst. Fiziol. Rast. AN SSSR 5, No. 2 (1947).
2. N. A. Maksimov, Selected Works on Plant Drought Resistance [in Russian] (Izd. An SSSR, 1952) Vol. II.

EFFECTS OF THE LONG DAY ON SUGAR NITROGEN CONTENT OF THE ONION PLANT

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Despite the appreciable amount of work devoted to the question of the biochemistry of the onion plant [1-6], fundamental to bulb formation and the reactions of the plant to photoperiod, it remains an unresolved problem at this time. Among works in this direction is necessary to mention the investigation of Reimers [7,8] and his co-workers [9].

In our work with the onion Allium cepa we tried to reveal the factors which contribute to the formation and normal growth of the onion, specifically under the stern conditions of the Polar North, and the significance of the polar day not only for the direction of the growth and development of plants but also for the trend and rate of biochemical processes which occur in the onion plant during the formation of individual organs. With this in mind we conducted experiments which included tracing the formation and accumulation of sugars in plants, changes in the amounts of different nitrogen fractions, and accumulation of dry matter during growth and formation of onion during a long natural day and a short day.

The work was done in the Polar-Alpine Botanical Garden and on the experimental plots in Apatity (67° N) of the Kol'skii Affiliate of the Academy of Sciences of the USSR, under the direction of Professor Z. I. Zhurbitskii. The onions were raised in greenhouses and hotbeds maintained with various temperatures and light regimes. One group of plants was held in a long natural day* and another group was subjected to an 11-13 hour day (by covering the frame with a black cover) during the initial period of plant growth (a short day produced by covering for 4 hours on June 23). Part of the plants on long days and short days were raised at an elevated temperature and another part at a lowered temperature. In the first case, the minimum temperature fluctuated from 6.5 to 14°C and the average daytime temperature from 11-24°C. In the second case, the minimum temperature was held from 3-4° to 9° and the average daytime temperature ranged from 8 to 21°.

The data in Tables 1-4 show the close relationship between the effect of external environmental factors and onion bulb formation on the one hand, and metabolism on the other.

When the first samples were taken at the end of June the plants on long days had already begun to form bulbs. At this time there were still no bulbs in the plants subjected to short days. Correspondingly, plants receiving short days contained appreciably less sugar in the

* The natural day in May consisted of 17-22 hours, in June 23-24 hours, in July 24-19 hours, in August 18-15 hours.

TABLE 1. Changes in the Sugar Content and Increase of Dry Weight of Strigunovskii Onion at Various Day Lengths and Elevated Temperatures (% of dry weight)

Day length	Date sampled	Bulb or leaf base				Leaves			
		dry wt/ 100 plants	monosaccharides	sucrose	total	dry wt/ 100 plant	monosaccharides	sucrose	total
Short	27.VI	1.31	12.56	23.66	36.22	7.75	13.74	8.67	22.41
Long		3.23	11.58	44.34	55.92	6.33	19.90	7.84	27.74
Short	4.VII	3.45	10.88	48.73	59.61	11.26	Not analyzed		
Long		7.08	7.60	53.33	60.93	8.43	17.65	10.17	27.82
Short	27.VIII	69.17	5.44	65.64	71.08	11.88	4.66	3.21	7.87
Long		35.84	6.16	71.35	77.51	Leaves died			

TABLE 2. Sugar Accumulation in Strigunovskii Onion at Various Day Lengths

Day length	Date sampled	Bulb or leaf base			Leaves		
		dry weight/ 100 plants	monosac-charides	sucrose	dry wt/ 100 plants	monosac-charides	sucrose
Short	27.VI	0.164	0.310	0.474	1.065	0.671	1.736
Long		0.374	1.432	1.806	1.260	0.496	1.756
Short	4.VII	0.375	1.681	2.056	Not analyzed		
Long		0.460	3.776	4.236	0.488	0.857	1.345
Short	27.VIII	3.763	45.403	49.166	0.554	0.381	0.935
Long		2.208	25.572	27.780	No leaves		

TABLE 3. Changes in Various Nitrogen Fractions in Strigunovskii Onion at Various Day Lengths (in percent of dry weight)

Day length	Date sampled	N, whole plant			N, bulb or leaf base			N, leaf		
		pro-tein	non-pro-tein	total	pro-tein	non-pro-tein	total	pro-tein	non-pro-tein	total
Short	27.VI	2.37	0.20	2.57	1.77	0.31	2.08	2.47	0.18	2.65
Long		1.66	0.34	2.00	0.84	0.22	1.06	2.08	0.40	2.48
Short	4.VII	1.91	0.34	2.25	1.87	0.21	1.08	2.32	0.40	2.72
Long		1.48	0.33	1.81	0.66	0.17	0.83	2.20	0.47	2.67
Short	27.VII	0.88	0.33	1.21	0.84	0.37	1.21	1.46	0.31	1.77
Long		—	Her	—	0.92	0.35	1.27	Not analyzed		

basal part of the onion leaves than did onions growing in uninterrupted natural days. However, after a certain time, about July 4, because of sunny, warm weather, short-day plants also began to form bulbs. This is accompanied by an abrupt increase (36-59%) in the sugar content of the basal part of the leaves (Table 1).

During this period, although the amount of sugar increases in bulbs of long-day plants, it is appreciably less (5%) than in short-day plants. Changes in the sugar content occur primarily because of changes in sucrose content. Toward the time the onion ripens the percent sugar in the bulb reaches 77.5% in plants on uninterrupted long days. A decrease in sugar content of leaves is observed during vegetative growth. In raising the onion, during the initial growth period at decreased temperatures the formation and accumulation of sugars in short-day plants is slow and the bulb forms correspondingly slower, and at maturity the difference between the sugar content of plants under short- and long-day treatment is obliterated. Thus, from June 27 to July 4 the sugar content of short-day plants climbs from 36.45 to 51.82%, while in long-day plants the sugar content on July 4 has already reached 63%. However, toward the end of the vegetative period the total sugar content (61-63% of dry weight) in these treatments is equalized but it does not attain the same high value observed in plants grown at elevated temperatures. Likewise, in plants raised in covered beds, the percent sugar in all cases fluctuates from 60-65% of dry weight.

From Table 2 it is apparent that, in agreement with the increase in percent sugar in long-day plants, an increase occurs in the total amount of sugars both in short- and long-day plants, but at different rates.

Interesting changes occur in the amount of nitrogen compounds in onion plants in relation to day length. Data in Table 3 show that during bulb formation the percent protein and total nitrogen decreases in the basal parts of the leaves. This is evident by comparing the data for long- and short-day plants for June 27: An abrupt decrease in the nitrogen content of the leaf bases occurs for a short period (about July 4) in short-day plants during bulb formation. The amount of leaf nitrogen decreases somewhat during the vegetative development under long day conditions.

Toward the end of the vegetative period, depending on the growth of the bulb, the amount of nitrogen in the bulbs increases somewhat, presumably in connection with the formation of leaf primordia. Bulb formation in plants transferred from short to long days takes place with a somewhat reduced rate of uptake and accumulation of nitrogen in the basal part of the plant (up to July 4); but about August 27, because of accelerated growth, the plants at elevated temperature accumulate more nitrogen than plants remaining continuously under long-day conditions.

TABLE 4. Changes in Uptake and Accumulation of Total Nitrogen in Strigunovskii Onion at Various Day Lengths (g/100 plants)

Day length	Whole plant	Bulbs or leaf bases	Leaves
		27.VI	
Short	232.6	27.2	205.4
Long	191.2	34.2	157.0
		4.VII	
Short	343.6	37.3	306.3
Long	283.9	58.8	225.1
		27.VIII	
Short	1066.1	836.9	229.2
Long	—	455.2	Not analyzed

In treatments at lowered temperature there is a greater total amount of nitrogen not only in the basal parts but also in the entire plant which received short days. Total nitrogen was less than that in plants which remained under long-day conditions for the entire period (Table 4).

With age the long- and short-day plants differed in their nitrogen uptake. The average rate of nitrogen uptake and accumulation was less in long-day plants, which may be linked with a difference in the nature of the growth processes.

From the data presented it is clear that the rate of bulb formation depends closely upon the rate of sugar formation and accumulation and the rate of nitrogen uptake and accumulation, which is closely linked to the effect of factors of the external environment (day length, and temperature). Under our conditions, a quite rapid reaction of onion plants to alterations in day length was observed.

In plants grown continuously under long natural days the percent nitrogen content was lower, while the percent sugar and the rate of its formation and accumulation were greater than in short-day plants. Leaf growth was quickly curtailed and bulbs were formed rapidly in long-day plants at elevated temperatures. The rate of nitrogen uptake and accumulation was different in the various stages of plant development, depending not only upon day length but on temperature effects as well.

Short days stimulated leaf formation and delayed bulb formation.

When plants were shifted from short to long days, the rate of sugar formation and accumulation increased, the amount of sugars in the plants began to increase rapidly, the percent sugar content rose, particularly during bulb formation, while the percent nitrogen in the bulbs rapidly fell simultaneously with a certain lag in nitrogen uptake and accumulation. With a temperature increase the amount of nitrogen accumulated by short-day plants was greater than by plants uninterruptedly under long days. Under these conditions the bulbs form rapidly. The greater the age of the bulb in short-day plants, the more the rate of sugar formation and accumulation accelerated, and the total amount of them became greater than in continuously long-day plants. This is related to the fact that in long-day plants the strong effect of an uninterrupted day and elevated temperature (during the initial growth period) is rapidly curtailed by the growth processes and the onset of bulb maturity. As a result plants subjected to short-day influences for 20-25 days, other conditions being equal, produce bulbs of larger diameter and weight than plants kept continuously under long days.

It is essential to note the positive consequences of a lowered temperature upon the formation and growth of the bulbs which are much heavier and larger than those subjected (during the initial growth period) to the comparatively disruptive effect of a short day at a lowered temperature. In experiments conducted under other meteorological conditions the weight and diameter of the bulbs and also the entire plant was two to three times greater but the differences, depending on the effect of day length and temperature, were the same.

LITERATURE CITED

1. Z. I. Zhurbitskii, Conference on Biochemistry of Fruits and Vegetables [in Russian] (Izd. AN SSSR, 1955) Vol. 3.
2. V. V. Arasimovich, Biochemistry of Crop Plants [in Russian] (Sel'khozgiz, 1948) Vol. 8.
3. V. V. Arasimovich, Trudy po Prikladnoi Botanike, Genetike i Selektsii 28, No. 3 (1950).
4. B. A. Rubin and V. E. Trupp, Trudy po Prikladnoi Botanike, Genetike i Selektsii 3, No. 15 (1936).
5. A. P. Shivrina, Biochemistry of Crop Plants [in Russian] (1938) Vol. 4.
6. E. M. Azarevich, Doklady VASKhNIL, No. 3 (1951).
7. F. É. Reimers, Abstracts of Papers of Conference on Plant Physiology [in Russian] (Izd. AN SSSR, 1940).
8. F. É. Reimers, Growth, Morphogenesis and Phasic Development of the Onion Plant (Dissertation) [in Russian] (1956).
9. L. N. Gordon, Effect of Day Length on Onion Growth and Yield. Abstracts of Dissertations [in Russian] (1952).

EARLY PRODUCTS OF PHOTOSYNTHESIS AND METHODS FOR ISOLATING THEM

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The question as to the nature of the first products of photosynthesis recently has been a source of lively discussion in biochemical literature. Precisely, it consists of two points of view: according to one, the initial product is phosphoglyceric acid [1] and according to the second, an oxy-acid of more complex structure [2,3]. Such a divergence of views could be a result of the different methods employed by the two groups of workers for the fixation and isolation of the first products of photosynthesis.

By avoiding the effect of high temperature during the separation process (by fixation with liquid nitrogen and infrequent handling of the material at 0°C) it has been possible to show that the largest part of the activity resides in the acids which precipitate in alcohol [3].

In as much as these data are at variance with those obtained by other workers [1] it is of interest to compare the initial products of photosynthesis in the same plants fixed and isolated by various methods. We used Primula obconica leaves in this investigation.

Short period photosynthesis was accomplished in a chamber modified from the one employed earlier [3]. The chamber with a gas volume of 104 ml contained 0.3 vol. % of carbon dioxide with a total activity of 70.2 μC. Photosynthesis was carried out with 500 w reflector lamps fixed at a distance of 25 cm. Leaves were exposed in the chamber for 20, 5, and 2 sec.

In the first part of the work (20 sec exposure) two methods of fixation and extraction were compared. First, Benson's method [4] where the leaves were fixed and extracted with 80% and 20% boiling ethyl alcohol, and boiling water, each time for thirty minutes, and then washed with water. Extracts were combined. In another case the leaves were fixed by killing in liquid nitrogen with two fold extraction with 10% cold trichloroacetic acid for three hours and subsequent washing with 10% trichloroacetic acid.

In order to study the extracted substance we used radiochromatography [5,6]. Extracts unconcentrated or precipitable in 60% acetone were applied to the paper (chromatographic paper from the Leningrad paper factory) and dried in a stream of cold air and chromatographed three times by the "sector" method [5] with a mixture of butyl alcohol, acetic acid and water, 4 : 1 : 5. X-ray film XX was used to obtain the radioautographs.

The following indicators were used for identification of the substances partitioned on the chromatograms: bromophenol, ninhydrin, aniline phthalate, para-aminophenol, naphthorescinol, resorcinol, phosphorus developer, rhodamine, silver nitrate, iron developers etc.

In addition the chromatographs were scanned under ultraviolet light in a Brumberg ultrachemiscope.

We succeeded in establishing that radiochromatograms of alcohol and trichloroacetic acid extracts are both similar and distinctive (Fig. 1). They are similar in that the principal radioactivity is found in unstable phosphate esters. These esters are developed as weak acids with bromophenol and react with the alcohol groups and with iron. They do not react with sugars and amino acids.

Entry of the bulk of radioactivity into the unstable phosphate esters agrees well with the chromatograms of Metzner [7].

We made radiochromatograms of only three products extracted from the leaves (in the TCA extract they made up 98.6% of all C^{14}).

Since, according to Benson's method, an appreciable part of the C^{14} remains unextracted from the leaves (after 20 sec, 36.2%), this fact alone effects a difference in the product which appears on the radiochromatogram. This difference is especially clear in the original spots and slowly moves forward as a low radioactive zone. Products obtained by the Benson-Calvin method are absent on the chromatogram.

The slowly progressing radioactive zone, apparently, belongs to CO_2 fixed as an unstable complex. This zone reacts weakly with bromophenol and $AgNO_3$ and is very weakly absorbent in ultraviolet light.

What is particularly important and interesting is the appearance of a black zone of phosphoglyceric acid (PGA) on the radiogram of products isolated according

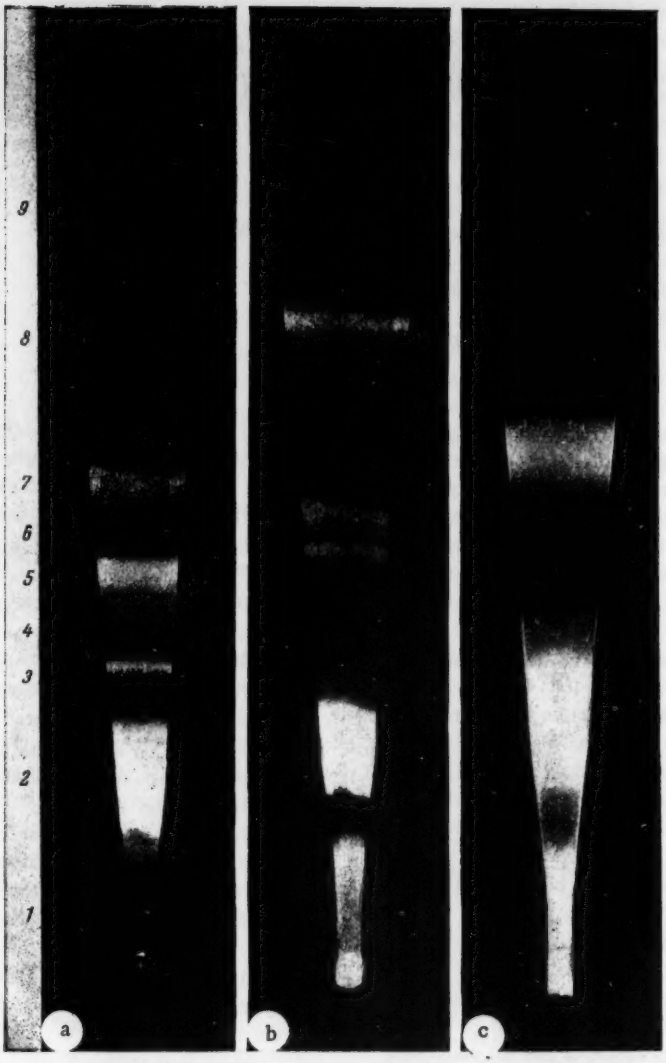

Fig. 1. Autographs of Radiochromatograms. Exposure 20 sec. a) Alcohol extract; b) trichloroacetic acid extract; c) acetone precipitate. 1) Complex; 2)phosphate esters; 3) phosphoglyceric acid; 4) carbohydrate; 5) acid; 6) carbohydrate; 7) ketose and amino acid; 8) glyceric acid; 9) malic acid.

to Calvin. This zone is absent on a radiogram of products obtained by fixing in liquid nitrogen.

The PGA was identified by the Rf of nonradioactive 3-phosphoglyceric acid obtained from the laboratory of B. N. Stepanenko; by its phosphorus reaction according to Khenes and Isherwood and by the reaction of bromophenol with the acid.

There is already an indication in the literature that the effects of alcohol misrepresent the results in the direction of PGA formation because the labile phosphate esters are dissociated [7,8,9].

The data presented are the result of a direct radiochromatographic analysis of products of boiling alcohol during work with the initial (unstable) products which decompose to form PGA.

Certain differences are also observed in the displacement of the migrating zones. Certain of them give a carbohydrate reaction. The principal interest here is the zone which gives both a carbohydrate and an amino acid reaction. Evidently, there is a superposing of zones and without a special study it is impossible to decide to which substance the radiocarbon belongs. The presence

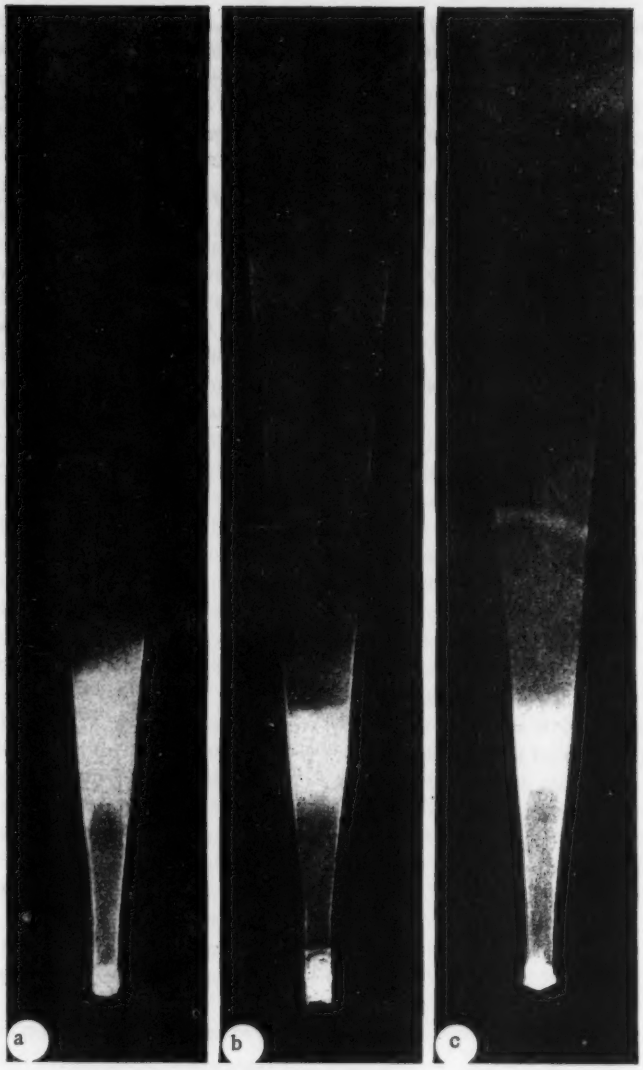

Fig. 2. Autographs of Radiochromatograms. Exposure 5 sec; a) Trichloroacetic acid extract; b) acetone precipitate; c) neutral precipitate.

of appreciable radioactivity has been described in a number of papers [10,11,12].

After the precipitation of the extract with 60% acetone, the acetone extract appears similar to the TCA extract. The acetone precipitation of substances containing a large part of the C^{14} indicates their complex nature.

When exposure is decreased from five to two seconds only the portions of the zones remain which correspond to the very earliest products of photosynthesis (Fig. 2).

The radiochromatogram of all TCA extracts after five seconds is reminiscent of the radiochromatograms of substances precipitated by acetone after a 20 sec exposure. Almost all of the C^{14} in the five second exposure is found in the phosphate esters precipitable in acetone.

It was of interest to fractionate the other substances from the acetone precipitate in order to characterize them. For this reason the entire precipitate was extracted with TCA, neutralized, and a second precipitation made with 60% acetone.

Again on the radiochromatogram a zone of oxy-acid was obtained containing phosphorus and iron and giving a reaction with $AgNO_3$. This zone always appeared on all radiochromatograms. It is natural to suppose that this oxy-acid is the initial product of photosynthesis.

In conclusion, I would like to express my deep appreciation to E. A. Boichenko for directing this study.

LITERATURE CITED

1. M. Calvin, J. M. Anderson, J. A. Bassham, U. Blass, O. Holm-Hansen, V. Moses, N. G. Pon, P. B. Sogo, and G. Tollin, Photosynthesis. Second United Nations International Conference on the Peaceful Uses of Atomic Energy (Geneva, 1958).
2. E. A. Boichenko and N. I. Zakharova, Biokhimiya 21, 374 and 623 (1956).
3. A. M. Kuzin and G. N. Saenko, Biofizika 11, 313 (1957).
4. A. A. Benson, Moderne Methoden der Pflanzenanalyse (Berlin, 1955) Vol. 11, p. 113.
5. R. J. Block, E. L. Durrum, and G. Zweig, A Manual of Paper Chromatography and Paper Electrophoresis (New York, 1958).
6. I. M. Hais and K. Macek, Handbuch der Papierchromatographie (Grundlagen und Technik, Yena, 1958) Vol. 1.
7. H. Metzner, Biol. Zbl. 77, 5, 513 (1958).
8. O. Kandler, Z. Naturforsh, 13B, 4, 219 (1958).
9. N. I. Zakharova, Fiziol. Rastenii (in press).
10. L. A. Nezgovorova, Fiziol. Rastenii 3, 497 (1956).
11. O. Warburg, Science 128, 3315, 68 (1958).
12. I. A. Tarchevskii, Photosynthetic Products of Wheat Leaves and the Effect of Edaphic and Atmospheric Drought on Their Formation Authors' Abstracts of Dissertations [in Russian] (Kazan, 1957).

EFFECT OF LIGHT AND TEMPERATURE ON BIOSYNTHESIS OF INDOLE TYPE ASCORBIGEN

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Since the first data were obtained [1-3] on the linked form of ascorbic acid in plant material, investigations of this oxidation-resistant form of vitamin C, so-called ascorbigen [3], have moved ahead appreciably [4-6].

Irrefutable evidence of the existence of ascorbigen in cabbage was obtained by Procházka and Kořistek [7]. Chemical research on ascorbigen culminated with its isolation [8] and a proposed possible structural formula [9] as ascorbic acid linked with 1 (3-indolyl)-propene-2-diol-2,3.

Investigation of the metabolism and function of ascorbigen began with a study of its distribution and translocation in individual organs of cruciferous plants during the vegetative period [10-15]. It has been established that ascorbigen is found in a majority of cases in the stem, growing tip and the young leaves. With tissue aging the amount of ascorbigen decreases. Ascorbigen is found in large amounts in young, rapidly growing plants; on the basis of its distribution and pattern of biosynthesis ascorbigen is similar to natural indole derivatives and free ascorbic acid.

In our present work we made determinations of ascorbigen formation in individual organs of cruciferous plants grown under field conditions. The determinations were made by the colorimetric method we have proposed [13]. After ascorbigen was separated from the natural products of indole it was determined chromatographically. Plant samples were taken every six hours.

In Fig. 1 are shown the changes in ascorbigen content of cabbage plants during a sunny four-day period with uniform weather (average night temperature about 12°, day temperature 20°). The ascorbigen content goes through periodic maxima which appear especially sharp in young, growing tissues of plants, in which the ascorbigen concentration is observed to the greatest. The ascorbigen content is not uniform in all plant organs and decreases in the following order: stem tips with young leaves > young leaves > older leaves > old leaves > center of the stem > roots. The ascorbigen content increases during the day and decreases at night. A corresponding periodic fluctuation in ascorbigen content is also observed in plants of Brussels sprouts of varying ages [12]. From this it is evident that the periodic fluctuations in young leaves and the stem tip are not analogous to changes occuring in other parts of the stem. On this basis it can be suggested that biosynthesis and destruction of ascorbigen occurs directly in organs of the plant [9,11].

We proposed that the indicated periodicity of ascorbigen accumulation can be caused by light and temperature changes. We set up special experiments in order to test this hypothesis.

Five-week old Kohlrabi plants grown in beds were held in the dark or light for two days at various temperatures (Fig. 2). The ascorbigen content appeared different at different temperatures. It was established that the optimal temperature for ascorbigen biosynthesis was 22°C.

The plants responded to the temperature changes initially by rapid changes of ascorbigen content which

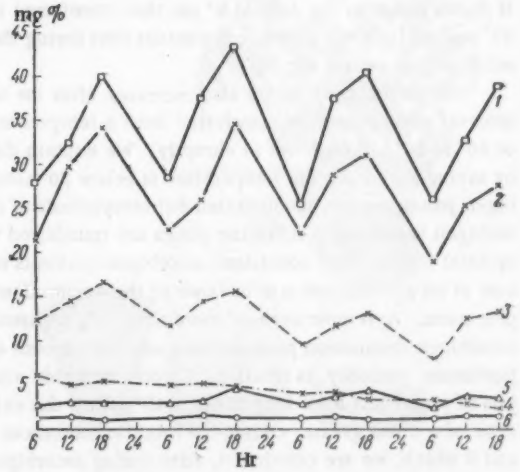

Fig. 1. Changes in the ascorbigen content of cabbage every six hours during four warm sunny days (mg/g dry weight). 1) Stem tip and youngest leaves; 2) young leaves (5, 6); 3) older leaves (3, 4); 4) old leaves (1, 2); 5) stem; 6) roots.

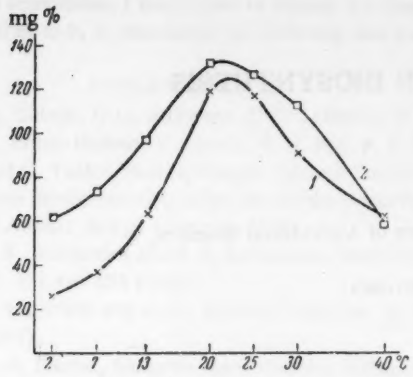

Fig. 2. Effect of temperature and light on ascorbigen content in tips of Kohlrabi plants (mg % dry weight). 1) Stem tips and youngest leaves; 2) young leaves (5, 6).

later decreased and after 30-46 hours the amount of it in the plant remained constant.

In Fig. 2 the effect of light on ascorbigen formation is also apparent. Plants grown in the dark always contained less ascorbigen than illuminated plants. The ascorbigen content also depends upon the light intensity. Changes in ascorbigen content caused by a dark period are reversible. The ascorbigen content of etiolated plants also increased in the light.

The effect of the two factors temperature and light together was revealed by certain combined experiments. When plants are grown in the dark at 2° and then transfered to the dark at 20° C the ascorbigen content in the stem tip and young leaves rises from 2.8 to 16.2 mg %. If plants raised in the dark at 2° are then transferred to 20° and sunlight the ascorbigen content rises during the same period to 31.4 mg %.

The ascorbigen content also increases after the same interval when plants are transferred from a temperature of 40° to 20°, although not so abruptly. We explain this by saying that when the temperature is below 20° ascorbigen precursors are accumulated but biosynthesis of ascorbigen is retarded. After the plants are transferred to optimal temperature conditions ascorbigen synthesis occurs at an accelerated rate because of the accumulated precursors. At temperatures of more than 22°, apparently, ascorbigen destruction predominates and the amount of precursors probably is smaller. Chromatographic analysis of plants left for a long time at 40° affirm the existence of a much greater content of specific substances T and S which, we are convinced, arise during ascorbigen destruction in detached cabbage leaves [13].

It is necessary to examine the experiments conducted with pure ascorbigen isolated by Procházka and Šǎnda [8,9]. Pure ascorbigen likewise was exposed to various high temperatures (up to 100°). Dry ascorbigen was most resistant. The stability of ascorbigen in solutions decreased depending on the temperature and the polarity of the solvent. Ethyl acetate solutions of ascorbigen are more stable than ethanol solutions, and its stability is somewhat less in solutions buffered at pH 5.5 Ascorbigen is least stable in distilled water (at 100° it decomposes completely in two hours).

Dry ascorbigen is photostable. A buffered solution of ascorbigen (pH 4-6) decomposes only 20% in six hours under ultraviolet light (Filora 125 v). Within other pH limits ultraviolet light strongly disintegrates ascorbigen.

From the results presented the fact emerges that temperature and light are important external factors influencing the ascorbigen level in cruciferous plants.

The following conclusions can be drawn from the research presented:

1. Ascorbigen biosynthesis proceeds rapidly in young leaves and stem tips. In these parts of the plant there is a very clearly expressed periodicity in this biosynthesis.

2. Light and temperature exert an appreciable effect on ascorbigen biosynthesis in plants. Ascorbigen biosynthesis accelerates when plants are transferred from dark to light. Optimal temperature for biosynthesis is 22°.

3. Ascorbigen in solution form is less stable to various influences than is the dry preparation.

LITERATURE CITED

1. N. Bezssonoff, Bull. Soc. chim. biol. 16, 1107 (1934).
2. E. W. McHenry and M. L. Graham, Nature 135, 871 (1935).
3. B. C. Guha and J. C. Pal, Nature 137, 946 (1936).
4. B. M. Sumtsov, Biokhimiya 15, 112 (1950).
5. E. L. Terent'eva, Biokhimiya 18, 296 (1953).
6. E. A. Chikalova, Fiziol. Rastenii 6, 724 (1959).*
7. Ž. Procházka and S. Kořistek, Chem. listy 45, 415 (1951).
8. Ž. Procházka and V. Šǎnda, Chem. listy 52, 237 (1958).
9. Ž. Procházka, V. Šǎnda, and F. Šorm, Collection, 22, 654 (1957).
10. M. Valenta, M. Kutáček, and F. Icha, Sbornik ČSAZV, Rostlin, 29, 193 (1956).
11. M. Kutaček, M. Valenta, and F. Icha, Experientia 13, 284 (1957).
12. M. Valenta, M. Kutáček, F. Icha, L. Holková, Sbornik ČSAZV, Rostlin, 31, 1579 (1958).
13. M. Kutáček, M. Valenta, and F. Icha, Collection, 24, 285 (1959).
14. M. Kutáček, F. Icha, M. Valenta, J. Tupý, and L. Holkova, Collection 24, 817 (1959).
15. M. Valenta, L. Holkova, V. Sanda, and M. Kutáček, Collection 1. State Conference of the Czechoslovakian Biochemical Society (Prague, 1957) p. 103.
16. M. Kutáček, M. Valenta, and J. Novakova, Collection (in press).

*See English translation.

USE OF GRAVEL CULTURE IN THE STUDY OF SOIL AND ATMOSPHERIC DROUGHT

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Today, a wide variety of methods are available to the plant physiologist studying and analyzing plant resistance to moisture deficiency and overheating [1]. One of the methods widely used for the study of plant drought resistance is the wilting method proposed by Tumanov [2,3]. In this method the plants are raised in soil in the greenhouse. Drought is achieved usually by cutting off irrigation for a few days. In a number of physiological experiments with drought it is very important to create sudden edaphic drought for a few hours in order to study the effect of atmospheric and edaphic drought on both the aerial and subterranean plant parts.

All of the above requirements have been incorporated in our modification of the gravel culture method which we are describing here.*

We made the following modifications in the gravel culture technique in order to use it in the study of drought effects. Since the plants were raised indoors [4,5] we used two vessels. One of them, a clay pot, was first filled with pea gravel. The second vessel was an ordinary glass jar used for water in soil-culture of plants. The glass jar, wrapped with paper, was used for nutrient solution and was fitted with a special aluminum ring in order to hold the clay pot half submerged in the nutrient solution (Fig. 1).

Gravel is convenient as an artificial medium for raising plants for it prevents the root system from drying out and furnishes favorable aeration conditions (see Fig. 1). Water culture was first used in 1929 by Gericke of the University of California to produce greenhouse conditions which he called "Hydroponics." Later the water solutions were modified with gravel, sand, crushed rock, or fine cinders with periodic additions of nutrient solution or the dry nutrient salts. In the USSR, experimental rearing of plants on artificial media under commercial conditions was begun in 1939 by V. A. Chesnokov and his co-workers in the Botanical Institute of Leningrad State University.

Today, experiments on raising plants on artificial nutrient media are being conducted in the USSR, German Democratic Republic (East Germany), Netherlands, Sweden, England, India, Australia, and Japan. In Germany this method is being widely used in flower production [5]. To create soil drought, the inner clay pot filled with gravel is lifted out of the outer glass jar containing the nutrient solution and exposed for several hours. Wilting occurs after different intervals of time depending on the type of plant. To study atmospheric drought the two vessels are placed in a dry-wind cabinet so that the effect of atmospheric drought can be studied under optimal plant moisture supply.

The work was conducted with tomato plant variety Early Anna and sunflower variety Zhdanovskii 8281 with control plants also hardened to drought [6]. The tomato

Fig. 1. Experimental hydroponic setup for studying wilting.

*We offer our sincere thanks to Laboratory Technician N. D. Pronina who assisted in the work.

TABLE 1. Growth and Weight of Sunflower Plants (bud stage)

Treatment	Gravel culture		Soil culture	
	growth, cm	weight, g	growth, cm	weight, g
Unhardened	116.4	241.8	84.0	174.5
Hardened	120.7	259.7	89.8	193.2

TABLE 2. Yield of Early Anna Tomato in Sand and Gravel Culture

Treatment	Total fruit weight, g
Sand	
Control	978.0
Hardened	1559.6
Gravel	
Control	1230.6
Hardened	2241.4

seedlings were first grown in sand and were transferred to the gravel culture when they were two-weeks old. Seeds of sunflower and maize were sown into the gravel directly. The experiment was conducted using the nutrient mixture of Chesnokov and Bazyrina [7] as follows:

$Ca(NO_3)_2$	.50 g/liter
$NH_4(SO_4)_2$	.16
KNO_3	.40
$Mg\ SO_4$	.28
$KHPO_4$	.14
$FeCl_3$	.06
HBO_3	.07
$Mn\ SO_4$	.05
$Zn\ SO_4$	.01
$Cu\ SO_4$	.002

The nutrient was changed once each month and the amount of water lost through evapotranspiration was replaced daily so that the inside clay pot remained half submerged in the nutrient solution. These conditions for raising the plants in gravel culture are good with respect to aeration, i.e., half of the plant root system, including the root collar, is in the gravel and not in the nutrient solution. Good aeration and plant mineral nutrition have very beneficial effects on the plant growth and development as Table 1 shows.

We have experimented with the technique of filling the pot with sand instead of gravel, i.e., poorer aeration conditions. This very quickly depressed the growth and yield of the tomato plants (Table 2). It should be noted that the number of root hairs was appreciably greater in pots filled with sand.

Fig. 2. Effect of sudden brief edaphic drought on control (left) and hardened (right) Early Anna tomato plants

The possibility of increasing drought resistance by presowing hardening allows us to determine the range of drought tolerance of a plant species or variety [8]. The tomato pots were removed from the nutrient solution for six hours during the study of edaphic drought. In these experiments an abrupt fall in turgor was observed in unhardened plants while hardened plants remained still almost at full turgor (Fig. 2). Atmospheric drought was studied in a dry-wind cabinet at the Artificial Climate Station (phytotron) of the Institute of Plant Physiology. The air temperature varied between 40-45°C, the relative humidity 25-27% and wind speed 7-9 m/sec. A certain change in colloid-chemical characteristics was observed in the hardened plants; typically they had a much higher protoplasmic heat tolerance, elasticity, and viscosity (plasmolytic method) probably because of which they more easily withstood the edaphic soil drought and the combination of atmospheric and edaphic drought (Tables 3 and 4). The photograph (Fig. 3) shows the effect of two atmospheric droughts on the control and on the hardened tomato plants.

The hardened plants were also characterized by a more sturdy root system, i.e., an increase in total root volume and active absorbing surface, which in turn conferred a much greater endurance of drought (Table 5) and increased the yield of the hardened plants (2.24

TABLE 3. Protoplasmic Viscosity and Elasticity in Tomato Leaves (Early Anna Variety) After Edaphic and Atmospheric Drought (in min) Flowering Stage

Treatment	Before drought		After edaphic drought		After dry wind		Viscosity after dry soil and dry wind
	visc	elast	visc	elast	visc	elast	
Control	21	5	27	8	36	4	43
Hardened	25	6	30	11	37	7	48

TABLE 4. Heat Tolerance of Leaves of Early Anna Tomato

Treatment	Stage		
	1st true leaf	flowering	fruiting
Control	43	46	44
Hardened	47	48	49

TABLE 5. Volume, Total, and Active Absorbing Surface of Early Anna Tomato Roots (Flowering Stages)

Treatment	Before dry wind			During dry wind			After dry wind		
	vol.	total	active	vol.	total	active	vol.	total	active
Control	13.8	6.322	2.981	14.0	8.052	4.832	13.2	4.934	3.642
Hardened	15.3	9.464	5.224	15.8	10.842	5.246	14.6	7.234	3.846

kg) compared to the control (1.23 kg). With the method given it was very convenient to study the root system since the time required to wash out the root system was inconsequential and did not cause any of the damage associated with the prolonged washing that usually occurs in experiments where the plants are soil-grown. Moreover, new data concerning the role of the root system in metabolism, particularly its ability to synthesize amino acids, amides and proteins, permit us to consider study of the physiology of the root system as a very concrete objective of contemporary science concerning itself with the life of plants. In gravel culture it is convenient to study the effect of atmospheric drought on the synthesis activities of the plant root system. In our experiments the plants were subjected to dry winds for 6-8 hours at 42° C, after which the shoot was cut off and a glass tube was fitted onto the stump [9]. Samples of sap were taken for 24 hours. The effect of dry winds on the synthesis activity of the root system was calculated and also determined by analysis of the plant sap. By calculating the rate of exudation we detected (Table 6) that in sunflower plants raised in gravel culture the amount of exudate as well as the amount of mineral nutrient elements nearly doubled compared with plants grown in soil, which speaks for the favorable effect that gravel culture has upon the supply of mineral nutrient elements to organs of the shoot.

We studied the after-effects of dry wind on hardened and unhardened plants, i.e., it was interesting to elucidate how the synthesis activity of root systems, which had been interrupted by dry wind, was reestablished [9]. With this in mind we analyzed the plant sap two days after a dry wind (Table 7).

The data for Table 7 show that the influx of mineral nutrient elements rises quite appreciably in hardened

Fig. 3. Effect of two atmospheric droughts on control (left) and hardened (right) Early Anna tomato plants

TABLE 6. Exudation of a Single Sunflower Plant (ml/24 hrs)

Treatment	Conditions	Gravel culture			Soil culture		
		juvenile	budding	anthesis	juvenile	budding	anthesis
Unhardened	Control	9.50	19.21	12.12	3.37	9.64	5.0
	Dry wind	8.48	15.36	7.72	2.47	5.82	4.0
Hardened	Control	10.85	17.28	13.44	3.93	12.82	6.10
	Dry wind	4.35	11.64	9.30	1.57	4.67	3.05

TABLE 7. Aftereffects of Dry Wind on Sap Composition of Hardened and Unhardened Plants

Treatment	Rate	Loss of free organic acids	Less nitrogenous materials, % total nitrogen			Ratio, organic to mineral N
			NH_3-N	NO_3-N	amino and amide N	
Unhardened	10.20	1.23	4.05	43.9	47.9	1.60
Hardened	15.38	3.09	0.9	31.3	63.6	4.56

plants, from which we forsee, on the basis of the rate of sap exudation, an increase in the amount of free organic acids, a sharp increase in the amount of amino acids and amides, and in rate of conversion of mineral nitrogen compounds into organic. Consequently, in hardened plants there is a more vigorous recovery from dry wind damage to the synthesis activity of the root system than occurs in the unhardened plants.

SUMMARY

A simple modification of the hydroponics method for physiological investigation of the effect of soil and atmospheric water deficiency on plants is proposed by the authors (Fig. 1).

The effect of soil drought on presowing drought hardening is shown in Fig. 2. Unhardened plants lost their turgor, whereas the hardened ones retained it (Fig. 2). The aftereffect of a two-fold air drought on the yield of control (left) and hardened (right) plants is represented in Fig. 3. The modified method described here permits one to create soil drought in a short time (during several hours). This circumstance is very important for the solution of a number of problems pertaining to the physiology of drought resistance.

With gravel cultures it is possible to study the effect of atmospheric drought in the phytotron under conditions of normal or insufficient water supply (in particular simultaneous soil and atmospheric drought).

The method is very convenient for studying the synthesis activity of roots during droughts and also for studying the aftereffect of the latter. Finally, the effect of drought on the physiology of the root system can also be studied.

LITERATURE CITED

1. P. A. Genkel' Diagnosis of Drought Resistance in Crop Plants and Means of Increasing It [in Russian] (Izd. AN SSSR, 1956).
2. I. I. Tumanov, Trudy po Priklad. Bot., Genet. i Selek. 16, No. 4 (1926).
3. I. I. Tumanov, Trudy po Priklad. Bot., Genet. i Selek. 22, No. 1 (1929).
4. E. M. Tsvetaeva, How to Raise Vegetables and Flowers Without Soil [in Russian] Izd. "Sovet. Rossiya" (1957).
5. Grünert, Chr. Zimmerblumen. (Bauernverlag, Berlin, 1957) 4th Edition.
6. P. A. Genkel', Trudy Inst. Fiziol. Rastenii AN SSSR V, No. 1 (1946).
7. V. A. Chesnokov and E. N. Bazyrina, Vestnik Sel'sko-Khoz. Nauki (1957).
8. P. A. Genkel', Tezisy Dokladov Konf. po Fiziologiya Ustoichivosti (March 3-7, 1959).
9. I. V. Tsvetkova and I. N. Voronina, Tezisy Dokladov Konf. po Fiziologiya Ustoichivosti (March 3-7, 1959).

A METHOD FOR INVESTIGATING THE ABILITY OF PLANTS TO ENDURE WILTING

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The method proposed by Nichiporovich [5] has been used with great success in recent investigations of the comparative drought resistance characteristics of plants [1-4]. This method is seldom called a method for the determination of water-retention ability of plants. However, we ought to agree with Tyurina [6] that the water-retention ability of plants is that value determined by the suction force of the colloids of the cell protoplasm, since the rate of wilting and release of water by living leaves or shoots to dry atmosphere is an integral indicator of the manifold characteristics of plant drought resistance; the rate of water exchange [7], changes in the ratio of bound to free water [7], resistance to water deficiency [2, 3] etc.

In the present work we attempted to adapt the desiccator method of Genkel' [2] by construction of an apparatus which would allow us to follow with sufficient exactness the rate of water loss of those parts of plants placed in the desiccator or another chamber having constant atmospheric conditions.

We intended to combine the essentials of several methods in one apparatus: a) Genkel's method – determination of the ability of plant tissues to go without water; b) Nichiporovich's method – determination of plant water-retention characteristics; c) Smirnov's method – study of the rate of shrinking of leaf colloids; d) Kolpikov and Teterin's method – use of an optical tube, dynamometer, and mirror for exact determination of water loss; e) the possibility of adapting the apparatus to self-recording of the curve of change in plant tissue water content (after Kolpikov and Teterin).

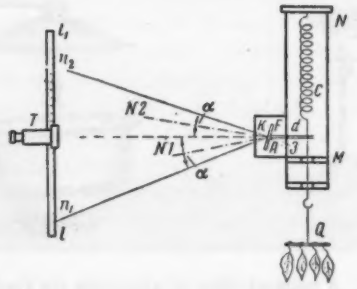

Fig. 1. Diagram of apparatus using a tube, mirror, and dynamometer (Description in text)

We based our method on the use of a desiccator, but the external apparatus can monitor any other chamber.

To obtain the intended resolution we used a sensitive spring dynamometer, mirror, and a telescope (Fig. 1). The dynamometer tube was mounted on the lid of the desiccator (Fig. 2). The method proposed is carried out as follows.

Assuming that the mirror surface A F (Fig. 1) is perpendicular to the plane of the sketch (Fig. 1), the line $n_1 n_2$ will be reflected from the mirror K onto the scale ll_1, which is immovably fixed on the telescope. A prolongation of the optical axis of the telescope intersects the plane of the mirror at point K. Lines Kn_1 and Kn_2 will be normal to the plane of the mirror AF. Under these conditions we will see in the telescope the image of the part of the scale $n_1 n_2$. The mirror AF rotates about an axis O perpendicular to the plane of the diagram (Fig. 1). The telescope hair fastened parallel to the axis of mirror rotation will coincide with n_1 in the starting position. After loading a weight Q onto the tube dynamometer, if we look through the telescope, the hair will fall on point n_2 on the scale. This occurs because under the influence of the weight the mirror alters its position. The mirror in rotating into a new position will move through the angle α and the hair will move to position n_2. The absolute difference in readings will permit us to follow quite accurately a rapid loss of water from the load in relation to time and experimental conditions. The apparatus itself is shown in Fig. 2. The results of one of these experiments is presented as an example in Table 1.

The data in Table 1 show that the differences in position of the hair on scale (See Fig. 1) ll_1 are directly related to the weight on the spring. As long as the position of the reflected image of the telescope hair on the scale is known, the loss of water from a

TABLE 1. Tabulation of the Position of the Ocular Hairs on the Scale with Various Weights on the Balance

No.	Mass, of wts, g	Initial reading	Final reading
1	0	0	
2	1	—	37.5
3	0	0	
4	0.5		19.0
5	0	0	
6	0.4		15.2
7	0	0	
8	0.2		7.9
9	0	0	
10	0.5		19.0

Note: The mass of weights was selected so that the elastic limit of the spring was not exceeded.

TABLE 2. Study of the Trend in Wilting of Leaves of the Middle Storeys of Capsélla Búrsa-pastoris Mnch. (per gram of leaves)

Reading	Time	Position of hair on scale
1	9:30 A.M.	37.0
2	9:42 A.M.	34.7
3	9:48 A.M.	33.7
4	10:00 A.M.	32.4
5	10:11 A.M.	30.4
6	10:40 A.M.	29.0
7	11:20 A.M.	26.0
8	1:25 P.M.	17.2
9	2:30 P.M.	13.5
10	3:00 P.M.	11.5
11	3:45 P.M.	9.1

plant sample will result in a corresponding shift in the image on the scale. It is simple then to graphically relate the differences in positions of the image on the scale to time during the experiment. Therefore, it is not necessary to periodically weigh the sample on an analytical balance as is done in the method of Nichiporovich [5].

The precision of our apparatus reaches 0.02 g in determining changes in the weight of sample losing water during wilting (with the scale one meter from the mirror). If the experiment demands greater precision, then the distance between the mirror and the scale must be increased.

Our apparatus may also be used for studying the rate of swelling of colloids if the sample is suspended in the dynamometer tube beneath the lid of a desiccator containing 10% H_2SO_4. Under such conditions the desiccator holds the relative humidity of the air inside at 96% [8]. The rate of weight increase as the colloid swells will be revealed by the position of the image of the hair on the scale. This allows us to readily graph the rate of swelling of plant tissue colloids. If, on the other hand, it is necessary to study the rate of change in the water-retention force of tissue colloids, the desiccator is first dried out with calcium chloride or other strong desiccants.

In order to study the weight of plants weighing more than one gram, it is necessary to have a dynamometer tube of somewhat larger diameter and a less flexible spring. Another variant is also possible: we can have a series of springs with various elastic limits of residual deformation. Then, depending upon the objective of the experiment we can insert various springs in the dynamometer tube and verify the readings of the apparatus as shown in Table 1.

Our apparatus permits a study of the rate of water loss by plants during the wilting process and under natural conditions. In this kind of study the apparatus (Fig. 2) is set up directly in the field without the desiccator. If weather (wind, rain, etc.) interferes with the observations it is necessary to put the support for the dynamometer tube into a plywood box and continue the observations. In experiments with Shepherd's-purse, Capsélla Búrsa-pastóris, during March and April 20, 1958 (maximum air temperatures 26.6°C, minimum −14°C, average relative humidity 45%, calm) we obtained the following data (Table 2) at Stavropol' in the Caucasus.

The method we have presented is more rapid, requires no tedious weighings on the analytical balance and subsequent calculation of the data into percentage and other comparative values. It directly obtains objective data for plotting curves of the trend in plant water loss during wilting. Not only that, but the method

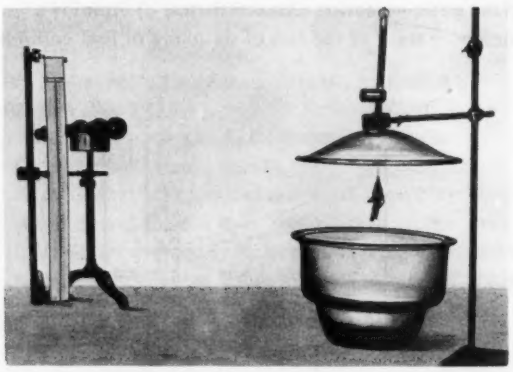

Fig. 2. A general view of apparatus for study of plant ability to endure wilting and tissue water stress and for determination of rate of tissue swelling after severe leaf wilting

makes it possible to more fully investigate this process not only in the laboratory but also under field conditions. Simplicity of construction and the possibility of using ordinary field binoculars as a telescope, lead one to hope for a wide use of our proposed method for a detailed study of the rate of water loss during wilting, the ability of plant tissues to swell in moist air, transpiration during wilting, and other processes taking place in plants both under natural conditions and in the controlled environment chamber.

SUMMARY

The desiccator method of Prof. Genkel' is extended by us. This is attained by using a sensitive spring dynamometer, a small mirror and a telescope (Figs. 1 and 2).

The tube with the dynamometer is fitted into the lid of the desiccator. Changes in the weight of samples of the plant rotate the axis of mirror F. Deviations of the mirror are reflected on the scale *ll*, and the wilting rate of the plant (sample) can be determined from the magnitude of the displacement. As described above our apparatus can be employed to determine the capacity of plants to endure dehydration (by the Genkel' method), to determine the water retaining capacity (by the Nichiporovich method), to study the dynamics of swelling of plant tissue colloids (by the weight method) and for determination of wilting transpiration.

By connecting the lever of the dynamometer spring with the axis of the recorder our apparatus can be used in various problems related to the wilting endurance of plants.

LITERATURE CITED

1. P. A. Genkel', Trudy Inst. Fiziol. Rastenii AN SSSR V, No. 1 (1946).
2. P. A. Genkel', Diagnosis of Drought Resistance of Crop Plants and Means of Increasing It [in Russian] (Izd. AN SSSR, 1956).
3. G. N. Eremeev, Abstracts of Papers of Conference on Physiological Resistance of Plants [in Russian] (Izd. AN SSSR, 1959).
4. L. G. Dobrunov, Physiological Changes During Plant Ontogeny [in Russian] (Izd. AN KazSSR, 1957).
5. A. A. Nichiporovich, Zhur. Optyn. Agronom Yugo-Vostoka 3, No. 1 (1926).
6. M. M. Tyurina, Fiziol. Rastenii 4, 378 (1957).*
7. D. I. Kolpikov, Trudy Stavropol'sk Sel'sk.-Khoz. Inst. No. VII (1956).
8. A. I. Smirnov, et al., Trudy Krasnodarsk', Inst. Tabakovodstva, No. 46 (1928).

*See English translation.

BOOK REVIEWS

N. S. Petinov, Physiology of Irrigated Wheat.
(K. A. Timiryazev Institute of Plant Physiology, Academy of Sciences, USSR) Izd. AN SSSR, 1959.
Reviewed by F. D. Skazkin.
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In a number of districts in our country there are periodically recurrent droughts in places where wheat, the most valuable grain crop, is produced. In arid districts with little precipitation which is irregularly destributed during the different growing periods, irrigation of agricultural crops is one of the most important measures of drought control. In some Far South districts agriculture is altogether impossible without irrigation.

The Party and the Government provided a number of measures for drought control from the very first days of the Great October socialist Revolution. Among these, irrigation occupies a most prominent place up to the present day.

Notwithstanding the fact that irrigation is one of the oldest agricultural problems, the application of irrigation in plant physiology was begun in the 1930's. The Soviet scientists were the pioneers in this field; they inaugurated the new sector of physiology – the physiology of the irrigated plant. One of the prominent places among these scientists is occupied by the author of the reviewed monograph.

The treatment of physiological problems in irrigated plants is of great import in establishing rational standards of hydration and the system of agricultural measures in irrigational environments.

Agricultural practice also presents problems to plant physiology of creating special physiology, i.e., physiology of individual crops. The reviewed monograph is dedicated to the physiology of the most valuable grain crop – that of summer wheat. The author and his coworkers in the laboratory under his leadership investigated the most important physiological functions of wheat; special attention was paid to the interaction of these functions in the organism as a whole. He accumulated during many years of his work in irrigational environments of Saratov, Kuibyshev, Kursk and Rostov regions, enormous, exceptionally interesting and valuable material on physiology of irrigated summer wheat, systematized and generalized in the monograph, the importance and timely publication of which leaves no doubt considering the statements above. The publication of this book is a great event in phytophysiology.

The reviewed monograph consists of two parts. The first part – "The ontogenetic development of wheat and value of water supply and mineral nutrient" is essentially devoted to the problems of biology and physiology of wheat development. The chief point in this portion of the monograph is devoted to revealing the value of water supply and mineral nutrient in wheat ontogenesis. The second part – "The effect of irrigation and mineral nutrient on the dynamics of the physiological processes, chemical processes and anatomical structure of wheat", a detailed summation, is devoted to the existing literature on these questions, also basic data by the author and his co-workers are stated on summer wheat physiology depending on one or another environments of irrigation. In concluding the second part of the study, the author poses the problem of clarifying the physiological properties of summer wheat varieties that are necessary for irrigation environments as well as establishing physiological characteristics in plants for irrigation requirements.

The first part of the monograph is interestingly conceived. It consists of four chapters:1) initial growth and development of summer wheat; 2) formation and differentiation of reproductive organs, growth of leaves, stems and spikes; 3) flowering; 4) ripening. In this part an almost exhaustive summation of summer wheat physiology is given. An attempt to create a similar summary was made in his time by I. M. Vasil'ev ("Studies on Wheat Drought-Resistance," 1929). In its time it was a study of great value. At this time, of course, that summary is obsolete. A. I. Nosatovsky's monograph on wheat biology (1950) contains little material on physiology of this crop. Thus, this part of the monograph can be considered unique.

In the first part of the monograph its author discusses step by step the physiology of summer wheat in its ontogenesis as well as the effect of factors in the external medium at different periods of its ontogenesis. This presents an absorbing interest for understanding how the crop's structure piles up in these circumstances from its separate elements, which is very important even to agricultural workers, especially to those who want to understand the mechanism of the yield, as related to precise agrotechnical measures, and want consciously to manage its creation. From this point of view a high evaluation should be placed on the first part of the author's study.

In the first chapter the author succeeds in giving a thorough biological basis to different methods of irrigation, norms and terms of watering based on wheat morphogenesis in its early stage of development. Morover, the author is not content merely with the morphological description of the root system, but he also broadly uses the physiological approaches in this field, such as: dissection of the root system into a general adsorbing and an active absorbing surface, the evaluation of the root system's role not only as an organ of water and nutrient substance absorption, but also as an organ possessing a high synthesizing capacity.

The center of attention in the second chapter is devoted to the extent and character of reproductive organ differentiation; also the question of leaf and stem growth is discussed. It is difficult to overestimate the importance of this chapter. In it the author successfully generalized his own numerous data, compared it with literature sources and as a result of painstaking study analyzed many important phenomena, among which we shall mention a very important one – plant drooping. The author discusses many theories of plant drooping, quite correctly indicates a unilateral approach of each, underlines the complexity of this phenomenon and very reasonably recommends the application of a complex of agrotechnical measures which includes rational irrigation methods, fertilizers, and planting methods, also the selection of varieties resistant to drooping.

The third and fourth chapters occupy relatively little space, but due to a definite compilation – they are devoted only to flowering and ripening – they also have significant interest. The reader will find original material here for the analysis of one of the most important phenomena in cultivation of bread grains – overgranulation, as well as grain sickliness.

Thus, vast data characterizing summer wheat in ontogenesis are given in the first part of the monograph.

Such a complete study of the growth and development of wheat closely related to agricultural ecology is advantageous to irrigated agriculture, where unlimited opportunities are created for managing the vegetating organism.

Despite all the evident merits in the above mentioned part, there are also defects.

In the discussion of some problems some individual studies are omitted. Thus, for instance, the author did not use the study of I. V. Krasovskaya on establishing a relation between the development stage of bread grains and emergence of leaves. No reference is made to some studies on the activity of trace elements in drought etc.

In the second chapter the significance of critical periods in plants is mentioned in irrigational agriculture environments. The author should have spoken not of critical periods, but of irrigation timing in wheat ontogenesis in relation to the need of creating the most favorable crop structure. There is one critical period in bread grains, while it is general in annual plants. Water deficiency can cause total spike sterility at periods from the beginning of maternal pollen cell formation up to flowering and fertilization inclusive. This is the critical period. This situation in plant ontogenesis is also vindicated from the evolutionary point of view. Therefore the critical period problem should have been referred to chapter 3 ("Flowering"), and treated more adequately since there are at present a number of interesting studies related to biology and physiology of flowering and fertilization of grains – and in relation to these the problem of the critical period should be treated in a modern sense.

These are the most pertinent remarks on the first part of the monograph.

The second part of the monograph – "Effect of irrigation and mineral nutrition on dynamics of physiological processes, chemical composition and anatomical structure of wheat" – consists of 5 chapters (5,6,7,8 and 9). In each chapter an interesting resume of studies is given.

In chapter 5 the author states in detail the results of studying the effects of irrigation and mineral nutrition on the plant's water regimen (total, free and combined, water plasma penetrability, its viscosity, suction force, osmotic pressure, transpiration,etc.), also on photosynthesis, respiration and increased growth of dry substance.

Because of broad coverage and meticulous care the author undoubtedly succeeded in presenting new concepts. He followed the daily progress of the most important function of leaves in a general manner and by analysis diagnosed the needed rate of providing plants with water as well as nutrient elements.

In the next chapter (6) the changes in the plants' chemical composition are studied as related to the different water and nutrient regimens. It is conclusively shown that a differential accounting of different forms of sugars and nitrogenous substances in their dynamics which allows a quantitative expression of the direction of synthetic and hydrolytic processes may serve as a method for evaluating the effectiveness, under definite conditions, of applied irrigation and nutrient regimens of plants. The theoretical and practical significance of these conclusions evidently need no explanation. A new concept is also promulgated by the author on the problem related to grain ripening physiology. It is known that in environments of high soil humidity the high synthesizing capacity of leaves notwithstanding, a relatively small quantity of protein substances is accumulated in grains. The author merits praise because not only did he explain the reason for this phenomenon, but he also devised and explained measures directed toward a higher protein content in wheat cultivated in irrigation environments.

Chapter 7 – "Changes in anatomical structure of wheat's leaves and stems," based primarily on original materials of the laboratory under the author's leadership

is valuable, among other things, by the fact that new data is promulgated on understanding anatomic features of wheat.

In drought the usual features of xeromorphic structure are acquired – chiefly adaptations which increase the flow of water are enlarged. However, in environments of irrigation and the use of mineral nutrient, mesomorphic features are intensified. And conditions can be created which contribute to a development of anatomic features in the plant related to an intensified synthetic activity which secures the most productive use of the increased quantities of water and nutrient substances in the soil. Guided by Michurin's studies the author established furthermore, that as a result of systematic plant cultivation with optimal supplies of water and mineral nutrient, these beneficial anatomic features become hereditary.

Chapter 8 – one of the important sections for selection. In this chapter, based on data in the literature and his own experiments, the author concludes that summer wheat varieties for environments of irrigated agriculture must have mesophyte features, and possess a special structure which provides large spikes with numerous ears. Moreover, the varieties must be resistant to high temperatures, a low relative air humidity, fungus diseases, etc. However, under conditions of hard water irrigation attention must be given to creation of transitional forms, and also, possible application of the chilling method to the seed material by P. A. Genkel' method. It is also important to select "seeds" for planting from irrigated sectors.

The value of the reviewed chapter consists in the fact that the author described a number of principles which can be utilized by selectors in their honorable trade of creating varieties for irrigation environments.

The concluding 9th chapter is devoted to physiological characteristics of irrigation needs by plants. The diagnosis of the functional plant condition, or, in K. A. Timiryazev's figurative expression, "requesting the plant's opinion," is, of course, one of the central problems of physiology as a biological basis of agronomy. It is commendable that precisely this problem, accurately completing the harmony, was sounded at the end of the monograph. One must agree with the author when he asserts that only characteristics of the plant's life activity (appropriate magnitudes of suction force, concentration of cellular juices, osmotic pressure, degree of stoma opening, determination of plants "crying need") permit rendering scientifically based and reliable methods of an early diagnosis of the plant's water needs. For such plants in concrete soil-climatic environments limits were established, on reaching of which irrigation is necessary. It shows how by taking advantage of these methods, water can be used economically and timed to obtain stable large yields.

A number of observations can also be made on the second part of the monograph. The author pays insufficient attention to plasma viscosity which can be one of the important characteristics of the plant's state and he has not utilized the literature in full measure on this problem. No attention was paid to the fact that the straw's conducting system is formed by leaves' or spikes' tracks. The structure of the bread grains' stalks should be studied from this point of view.

Despite the above mentioned remarks and suggestions, N. S. Petinov's monograph – "Physiology of irrigated wheat" – represents a valuable textbook for plant physiologists and the agrotechnical workers who desire to utilize scientific advancement in the practice of irrigated agricultural cultivations. Written in fine literary language, in a compact, coherently arranged style, carefully edited, the monograph extends far beyond the title given it by its author and essentially constitutes a resume of the basic principles of plant physiology problems in irrigation. Many cardinal problems of irrigated agriculture were put in a new light and based on the author's numerous field and laboratory experiments, such as the correlation of mineral nutrient and water supply as related to metabolism, changes in the water requirements by plants in accordance with age, changes in the crop's structure and quality in relation to the water supply, etc.

The monograph reflects the very obvious process occurring in modern times. The plant's water supply is no more limited to "water balances," "water metabolism," "water forms" in the plant, but is conceived in the light of the plant's processes of the total life activity, especially in its metabolism and regulatory relationships. Finally, the author does not confine himself to explanation of causes of undesirable phenomena, but offers rational methods of controlling them. This trait runs throughout the whole monograph like a red thread.

All this confirms that N. S. Petinov's monograph, into the creation of which the author invested enormous toil, is a very modern, original and new work and therefore is highly valuable.

The appearance of such monographs should be warmly welcomed.

B. A. Rubin, Lectures on Plant Physiology.
Moscow, 1959.
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Ten lectures are published in the book, given by the author to professors, instructors, and science fellows of universities and scientific-experimental institutions of the Chinese Peoples' Republic in 1957.

The author introduces the reader to a concept of plant physiology problems based on materialistic concepts, the unity of the organism and medium, the unity, or rather, the synchronization of universal evolution (medium) and plants. Proceeding from facts of diversity of functions in the plant at different epochs of the world's existence, the author approaches the establishment of a multiplicity of means for the realization of basic functions (respiration, enzyme interaction, photosynthesis and others) in different environments. The first lecture concludes with an exploration of modern problems of physiology which constitute the foundations of socialist agriculture. This chapter is written with originality and differs advantageously from similar introductions of other courses.

The second lecture is devoted to the adaptive role of physiological functions. Usually these problems are very poorly elaborated in textbooks. And yet they are exceptionally important for comprehending "nature, the so-called expeditious reaction to external activity" and "understanding the role of external medium factors in forming of metabolism characteristics peculiar to the organism." Rhythms in changes in the external medium (temperature, light, length of day, moisture, etc.) lead, in the long run, to an adaptation of metabolism such that the organism's life is made possible.

The third-fifth lectures are devoted to respiration problems. One must agree with the author that the exceptional complexity of enzymatic respiratory systems is related not only to the species composition but also to the plants' ecology, with the environments of its existence (favorable or unfavorable), with the state of the organism and other factors; this complexity is determined by the plant's adaptation to various factors of the external medium in which the plant exists, while perfection of the systems complexity consists of the most expeditious conversion of respiratory substances into the largest coefficient of its beneficial activity in each individual case. Numerous investigations by the author and his collaborators indicate that individual respiration stages - absorption of O_2 and liberation of CO_2 - are determined differently by external conditions; even separate tissues have different ratios of these stages. The optimal oxygen level is dissimilar for different tissues. Changes in temperature and a number of other unfavorable circumstances is also accompanied by changes in the ratio of respiration stages. This is explained by the fact that respiration is a complex process in which separate links are regulated by different enzymatic systems, which can undergo dissimilar changes.

In the fourth lecture the chemistry of the respiratory passage is treated: the dichotomous, achotomous and without preliminary phosphorylation, with an account of the latest achievements in this respect.

In the fifth lecture concepts are revealed of bonds in the heterogeneity of enzymatic systems of respiration and the "heterogeneity" of the medium; changes in the basic factors of the medium determining vital processes (of temperature, light, O_2 and CO_2 content during the ontogenesis period as well as the everyday one). In this connection, in higher plants many reactions are accomplished not by one enzyme, but by several simultaneously prevalent in the organism.

Approaching these problems from the evolutionary point of view, the author suggests that individual agents of the complex respiratory system are characterized by different activity depending on different conditions of the medium. As the medium changes, the enzyme also changes, namely: an enzyme best suited for the given environment enters the formation.

The author cites examples confirming that the basic role in the respiratory process is played by the enzyme for the activity of which the environments are nearest to optimal; changes in the individual protoplasm elements, on the other hand, contribute to the normalization of pathological processes due to the changes in correlation between the active and inactive portions of the enzymes.

The sixth-eighth lectures are devoted to the elucidation of physiology of interrelation between the higher plant and the parasite attacking it.

The author discusses the emergence of heterotrophs in evolution, problems of symbiosis and parasitism,

properties of obligative and facultative forms on a background of immune and nonimmune plant varieties.

Also the character of the parasite's activity (parasite's toxins and the plant's toxins formed as a result of the parasite's activity) or their component parts (ammonia etc.), and also the enzymes. The latter may greatly vary in activity depending on nature of nutrient intake, and in some cases they may generate anew. Great significance is ascribed to such adaptive enzymes. An interesting example is quoted from the studies of the Chinese scientist T'ang P'ei-sung, who found in higher plants the capacity to form adaptive enzymes (nitrate reductase in rice cultivated on media containing NO_3). In ending the chapter, the author concludes correctly that diseases arise as a result of existing corresponding conditions in the external medium, toxic substances of parasites and plants, arising in them because of metabolic anomalies, parasite and plant enzymes (activated through parasite activity), antibiotics, growth stimulants and other biologically active compounds and anatomophysiological properties of the plant-hosts. As an illustrative example, the activity is quoted of lycomarasmine and fusarinic acid (vilt), compounds with a specific ability to form metallochelates, to bind heavy metals and thus to disrupt the activity of oxidative enzymes.

The following lecture is devoted to the physiology of diseased plants. The author considers as of basic importance determining the character of the disease the following: 1) virulence of the provoking agent and the mode of its nutrition; 2) metabolic properties of plant-host, which determine its resistance; 3) character of mutual adaptation; 4) stage of disease; 5) external medium environments in which the parasite's attack occurs.

In relation to the disruption of the physiological processes in diseased plants, the author considers the following: a) increase of penetrability and exosmosis facilitating the formation of the "infectious drop," improving the parasite's feeding; b) changing the water supply-transpiration increase because of disturbance of covering tissues or disturbance of stoma movements by reason of inhibiting the synthesizing activity of phosphorylase through the effect of toxin in closing cells, while on the other hand, there is a decreased ability of the root system to absorb water owing to the plugging up of the conducting passages by the products of parasitic activity. Deterioration of the water supply causes an intensification of dissimilating processes, a decrease of chlorophyll and a decrease of photosynthesis intensity as well as a disruption in metabolic processes which is accompanied by an intensified activity of hydrolytic and other enzymes through the effect of toxin, and also the activity of enzymes of the parasite itself.

An actively destructive activity is exerted by such toxin components as ammonia (and amines of a simple structure) which can form due to amidase and urease activation.

In the eighth lecture of this series, the author proceeds to the accounting of the nature of internal defensive reactions, in particular to clarifying the role of respiration as one of the most important links in metabolism and at the same time one of the causes of immunity. At this time the author explains the meaning of changes that arise in establishing contact between the host-plant and the parasite. What should be noted in general is the activation of respiration in all cases of infection. The respiratory outburst in the host-plant is of short duration and the lower it is, the less the resistance; in resistant plants the struggle with infection is accompanied by intensified respiration for a long while. Intrusion of a parasite is accompanied by an essential reconstruction of the enzymatic system catalyzing the plant's respiration due to the difference in sensitivity to toxins of the individual elements of the system.

The role of the oxidizing system in immunity of plants consists of the following: a) oxidative and inactivating toxins; b) energy delivery for the support of normal (vital) cell structure; c) inhibiting hydrolysis of synthesized organisms; d) participation in synthesis of cell-wall elements etc. needed for creating barrier tissues.

The problem of obligative parasites has not been studied thoroughly. Here the author shares the opinion of so-called "tissue oversensitivity" to parasitic toxins and the destruction of these tissues together with the parasite.

There is a detailed discussion of the enzymatic mechanism of "oversensitivity" disclosed by the author in a joint study with Aksenova.

Based on the concept of the organism as a unit, the author in his ninth lecture discusses the role of roots in the plants' life activity. Beginning with I. V. Michurin who showed the effect of the root systems on formation of hereditary properties of grafted plants, the author thoroughly discusses the data in the literature on this problem, especially numerous researches with the use of graftings. The exceptionally important role of roots is illuminated not only in the synthesis of most important substances, but also of enzymes; in cases of grafting, the activity of root properties is observed on the graft's leaf apparatus.

In the last lecture the problem of quality and storage of vegetable material is discussed. Information is presented on improving the qualities of tea, tobacco, turnips, sunflower seed and other plants. The author also calls attention to the impairment of quality on disruption of metabolism, particularly in the shrivelling mosaic disease of potatoes.

Discussing the causes of unsatisfactory storage, the author points out chiefly the physiological diseases of

fruits which arise as a result of deviation from the normal course of metabolism (for instance, peel spottiness in citrus fruits, apple tarnish, pulp browning and others). Studies by the author and his collaborators helped to clarify the causes of these diseases.

The lecture concludes with consideration of the physiological action of hard γ-rays (Co^{60}) on stored potatoes. The inhibition of eyelet sprouting occurs because of a profound change in metabolism; a total regeneration of growth points sets in, their cells cease to divide, they protrude and vacuolate; the content of nucleic acids, which usually increases on the bulbs' emergence from the dormancy stage, on irradiation substantially decreases (to one half to one quarter) due to the intensification of ribonuclease activity. High radiosensitivity was also shown by oxidases found in the mitochondria; an increase in cytochromoxidase activity and a decrease in polyphenoloxidase and peroxidase activities. By varying environments the prospect of using γ-rays for potato lightness was successfully proven.

Regretfully, in some places the lectures are over simplified. Thus, in the first chapter the author states that "most investigators consider phosphoglyceric acid as the primary product of photosynthesis." However, based on Calvin's chart, cited by the author, the primary product is considered to be phosphoglyceric aldehyde. It should be added that in the complex of reacting systems acting on phosphoglyceric acid ATP, TPN, TPN-N_2 and dehydrases participate.

In the fourth chapter, on pages 80, 82 and 84 the diagrams should have been clearer. In some places the presentation is exceedingly niggardly (page 81, interpretation of data by Sleiter and others).

While mentioning that Maler and others have found copper content in flavoproteins (butyrin coenzyme A-dehydrase, page 80), it should have been mentioned that in applying trilone B, which extracts copper from this enzyme, its activity is unchanged (E. Steyn-Parvé, H. Beinert).

In presenting the oxidase system in potatoes (page 98) based on studies by Todt and the author, note should have been taken of the large amount of contradictory literature on this problem. Boswell and Whiting, Boswell, Walker and Nelson concluded that respiration of bulbs is 70% accomplished by polyphenoloxidase. Shade and Levy showed that 35-40% of respiration is accomplished at the expense of cytochromoxidase, and the remaining 60% is accomplished not at the expense of polyphenoloxidase, but that there must be one more metal-containing oxidase. According to James (and also Ivanova) it is ascorbinoxidase. Mul'der assumes that the final oxidase is neither polyphenoloxidase nor ascorbinoxidase. Goddard and Olden, also Barton, assume that independently active polyphenoloxidases and cytochromoxidases act in tubers. Timan and his coworkers exclude the possibility of polyphenoloxidase and ascorbinoxidase activity as the enzymes of the final respirational stage.

In the fifth chapter, Fig. 26, illustrating dynamics of dehydrases in larch and pine, is incorrect; three notations are interchanged. The knowledge of gibberellin activity at the present time is somewhat different than the one stated in the book: the most active form is gibberellic acid (A_3) and not gibberellin A (page 132). The interesting studies of Tomiyan on causes of potato immunity to phytofluor is little understood (pages 172-173).

In discussing his own interesting investigation of the characteristics of amylase activity on mosaic potato plants, revealing carbohydrate metabolism anomalies, the inversion of nitrogenous metabolism, characteristic of this disease, should have been dwelt upon, (page 204).

B. A. Rubin's lectures on plant chemical physiology present this branch of science well from the theoretical and practical view. The themes of the lectures are successful since they illuminate precisely those problems which are but slightly touched upon even in extensive physiology courses.

ANNOUNCEMENT

A bibliography of domestic and foreign literature on the subject of: "Photoperiodism of plants" for the period of 1951 to 1958 is compiled by M. V. Lomonosov Moscow State University. One may familiarize himself with the bibliography daily from 9 to 6 in the Scientific-bibliographical department of LMSU Library, Moscow, Mokhovaya, 9.

ABBREVIATIONS MOST FREQUENTLY ENCOUNTERED IN RUSSIAN BIO-SCIENCES LITERATURE

Abbreviation (Transliterated)	Significance
AMN SSSR	Academy of Medical Sciences, USSR
AN SSSR	Academy of Sciences, USSR
BIN	Biological Institute, Botanical Institute
FTI	Institute of Physiotherapy
GONTI	State United Sci-Tech Press
GOST	All Union State Standard
GRRRI	State Roentgenology, Radiology, and Cancer Institute
GTTI	State Technical and Theoretical Literature Press
GU	State University
I Kh N	Scientific Research Institute of Surgical Neuropathology
IL (IIL)	Foreign Literature Press
IONKh	Inst. Gen. and Inorganic Chemistry (N. S. Kurnakov)
IP	Soil Science Inst. (Acad. Sci. USSR)
ISN (Izd. Sov. Nauk)	Soviet Science Press
Izd.	Press
LEM	Laboratory for experimental morphogenesis
LENDVI	Leningrad Inst. of Dermatology and Venereology
LEO	Laboratory of Experimental Zoology
LIKhT	Leningrad Surgical Institute for Tuberculosis and Bone and Joint Diseases
LIPZ	Leningrad Inst. for Study of Occupational Diseases
LIPK	Leningrad Blood Transfusion Institute
Medgiz	State Medical Literature Press
MOPISh	Moscow Society of Apiculture and Sericulture
MVI	Moscow Veterinary Institute
MZdrav	Ministry of Health
MZI	Moscow Zootechnical Institute
LOKhO	Leningrad Society of Orthopedic Surgeons
NIIZ	Scientific Research Institute of Zoology
NINKhI	Scientific Research Institute of Neurosurgery
NIU	Scientific Institute for Fertilizers
NIUIF	Scientific Research Institute of Fertilizers and Insecticides
NIVI	Veterinary Scientific Research Institute
ONTI	United Sci. Tech. Press
OTI	Division of Technical Information
RBO	Russian Botanical Society
ROP	Russian Society of Pathologists
SANIIRI	Central Asia Scientific Research Institute of Irrigation
SANIISh	Central Asia Scientific Research Institute of Sericulture
TsNII	All-Union Central Scientific Research Institute
TsNTL	Central Scientific and Technical Laboratory
VASKhNIL	All-Union Academy of Agricultural Sciences
VIG	All-Union Institute of Helminthology
VIEM	All-Union Institute of Experimental Medicine
VIR	All-Union Institute of Plant Cultivation
VIUAA	All-Union Institute of Fertilizers, Soil Science, and Agricultural Engineering
VIZR	All-Union Institute of Medical and Pharmaceutical Herbs
VNIRO	All-Union Scientific Institute of Fishing and Oceanography
ZIN	Zoological Inst. (Acad. Sci. USSR)

Note: Abbreviations not on this list and not explained in the translation have been transliterated, no further information about their significance being available to us. - Publisher.

RUSSIAN JOURNALS FREQUENTLY CITED

[Biological Sciences]

Abbreviation*	Journal*	Translation
Agrobiol.	Agrobiologiya	Agrobiology
Akusherstvo i Ginekol.	Akusherstvo i Ginekologiya	Obstetrics and Gynecology
Antibiotiki	Antibiotiki	Antibiotics
Aptechnoe Delo	Aptechnoe Delo	Pharmaceutical Transactions
Arkh. Anat. Gistol. i Émbriol.	Arkhiv Anatomii Gistologii i Émbriologii	Archives of Antomy, Histology, and Embryology
Arkh. Biol. Nauk SSSR	Arkhiv Biologicheskikh Nauk SSSR	Archives of Biological Science USSR
Arkh. Patol.	Arkhiv Patologii	Archives of Pathology
Biofizika	Biofizika	Biophysics
Biokhimiya	Biokhimiya	Biochemistry
Biokhim. Plodov i Ovoshchei	Biokhimiya Plodov i Ovoshchei	Biochemistry of Fruits and Vegetables
Bot. Zhur.	Botanicheskii Zhurnal	Journal of Botany
Byull. Éksptl. Biol. i Med.	Byulleten Éksperimentalnoi Biologii i Meditsiny	Bulletin of Experimental Biology and Medicine
Byull. Moskov. Obshchestva Ispytatelei Prirody, Otdel Biol.	Byulleten Moskovskogo Obshchestva Ispytatelei Prirody, Otdel Biologicheskii	Bulletin of the Moscow Naturalists Society, Division of Biology
Doklady Akad. Nauk SSSR	Doklady Akademii Nauk SSSR	Proceedings of the Academy of Sciences USSR
Éksptl. Khirurg.	Éksperimentalnaya Khirurgiya	Experimental Surgery
Farmakol. i Toksikol.	Farmakologiya i Toksikologiya	Pharmacology and Toxicology
Farmatsiya	Farmatsiya	Pharmacy
Fiziol. Rastenii	Fiziologiya Rastenii	Plant Physiology
Fiziol. Zhur. SSSR	Fiziologicheskii Zhurnal SSSR im. I. M. Sechenova	I. M. Sechenov Physiology Journal USSR
Gigiena i Sanit.	Gigiena i Sanitariya	Hygiene and Sanitation
Izvest. Akad. Nauk SSSR, Ser. Biol.	Izvestiya Akademii Nauk SSSR, Seriya Biologicheskaya	Bulletin of the Academy of Sciences USSR, Biology Series
Izvest. Tikhookeanskogo N. I. Inst. Rybnogo Khoz. i Okeanog.	Investiya Tikhookeanskogo N. I. Instituta Rybnogo Khozyaistva i Okeanografii	Bulletin of the Pacific Ocean Scientific Institute of Fisheries and Oceanography
Khirurgiya	Khirurgiya	Surgery
Klin. Med.	Klinicheskaya Meditsina	Clinical Medicine
Lab. Delo	Laboratornoe Delo (po Voprosam Meditsiny)	Laboratory Work (on Medical Problems)
Med. Parazitol.	Meditsinskaya Parazitologiya i Parazitarnye Bolezni	Medical Parasitology and Parasitic Diseases
Med. Radiol.	Meditsinskaya Radiologiya	Medical Radiology
Med. Zhur. Ukrain.	Medichnii Zhurnal Ukrainskii	Ukrainian Medical Journal
Mikrobiologiya	Mikrobiologiya	Microbiology
Mikrobiol. Zhur.	Mikrobiologichnii Zhurnal	Microbiology Journal
Nevropatol., Psikhyat. i Psikhogig.	Nevropatologiya, Psikhyatriya i Psikhogigiena	Neuropathology, Psychiatry and Psychohygiene
Ortoped., Travmatol. i Protez.	Ortopediya, Travmatologiya i Protezirovanie	Orthopedics, Traumatology and Prosthetics
Parazitol. Sbornik	Parazitologicheskii Sbornik	Parasitology Collection
Pediatriya	Pediatriya	Pediatrics
Pochvovedenie	Pochvovedenie	Soil Science
Priroda	Priroda	Nature
Problemy Éndokrinol. i Gormonoterap.	Problemy Endokrinologii i Gormonoterapii	Problems of Endocrinology and Hormone Therapy
Problemy Gematol.	Problemy Gematologii i Perelivaniya Krovi	Problems of Hematology and Blood Transfusion
Problemy Tuberk.	Problemy Tuberkuleza	Problems of Tuberculosis
Sovet. Med.	Sovetskaya Meditsina	Soviet Medicine
Sovet. Vrachebny Zhur.	Sovetskii Vrachebnyi Zhurnal	Soviet Physicians Journal
Stomatologiya	Stomatologiya	Stomatology

*BRITISH-AMERICAN TRANSLITERATION SYSTEM.

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Abbreviation	Journal	Translation
Terap. Arkh.	Terapevticheskii Arkhiv	Therapeutic Archives
Trudy Gelmint. Lab.	Trudy Gelmintologicheskoi Laboratoriya	Transactions of the Helminthology Laboratory
Trudy Inst. Genet.	Trudy Instituta Genetiki	Transactions of the Institute of Genetics
Trudy Inst. Gidrobiol.	Trudy Instituta Gidrobiologiya	Transactions of the Institute of Hydrobiology
Trudy Inst. Mikrobiol.	Trudy Instituta Mikrobiologiya	Transactions of the Institute of Microbiology
Trudy Inst. Okean.	Trudy Instituta Okeanologiya, Akademii Nauk SSSR	Transactions of the Institute of Oceanology, Academy of Sciences, USSR
Trudy Leningrad Obshchestva Estestvoisp.	Trudy Leningrad Obshchestva Estestvoispytatelei	Transactions of the Leningrad Society of Naturalists
Trudy Vsesoyuz. Gidrobiol. Obshchestva	Trudy Vsesoyuznogo Gidrobiologicheskogo Obshchestva	Transactions of the All-Union Hydrobiological Society
Trudy Vsesoyuz. Inst. Eksptl. Med.	Trudy Vsesoyuznogo Instituta Eksperimentalnoi Meditsiny	Transactions of the All-Union Institute of Experimental Medicine
Ukrain. Biokhim. Zhur.	Ukrainskii Biokhimichnii Zhurnal	Ukrainian Biochemical Journal
Urologiya	Urologiya	Urology
Uspekhi Biokhimiya	Uspekhi Biokhimiya	Progress in Biochemistry
Uspekhi Sovremennoi Biol.	Uspekhi Sovremennoi Biologiya	Progress in Contemporary Biology
Vestnik Akad. Med. Nauk SSSR	Vestnik Akademii Meditsinskikh Nauk SSSR	Bulletin of the Academy of Medical Science USSR
Vestnik Khirurg. im. Grekova	Vestnik Khirurgii imeni Grekova	Grekov Bulletin of Surgery
Vestnik Leningrad. Univ. Ser. Biol.	Vestnik Leningradskogo Universiteta, Seriya Biologii	Journal of the Leningrad Univ., Biology Series
Vestnik Moskov. Univ., Ser. Biol. i Pochvov.	Vestnik Moskovskogo Universitera, Seriya Biologii i Pochvovedeniya	Bulletin of the Moscow University, Biology and Soil Science Series
Vestnik Oftalmol.	Vestnik Oftalmologii	Bulletin of Ophthalmology
Vestnik Oto-rino-laringol.	Vestnik Oto-rino-laringologiya	Bulletin of Otorhinolaryngology
Vestnik Rentgenol. i Radiol.	Vestnik Rentgenologii i Radiologii	Bulletin of Roentgenology and Radiology
Vestnik Venerol. i Dermatol.	Vestnik Venerologii i Dermatologii	Bulletin of Venereology and Dermatology
Veterinariya	Veterinariya	Veterinary Science
Vinodelie i Vinogradarstvo	Vinodelie i Vinogradarstvo SSSR	Wine-Making and Viticulture
Voprosy Klin.	Voprosy Klinicheskie	Clinical Problems
Voprosy Med. Khim.	Voprosy Meditsinskoi Khimii	Problems of Medical Chemistry
Voprosy Med. Virusol.	Voprosy Meditsinskoi Virusologii	Problems of Medical Virology
Voprosy Neirokhirurg.	Voprosy Neirokhirugii	Problems of Neurosurgery
Voprosy Onkol.	Voprosy Onkologii	Problems of Oncology
Voprosy Pitaniya	Voprosy Pitaniya	Problems of Nutrition
Voprosy Psikhologii	Voprosy Psikhologii	Problems of Psychology
Voprosy Virusologii	Voprosy Virusologii	Problems of Virology
Vrachebnoe Delo	Vrachebnoe Delo	Medical Profession
Zav. Lab.	Zavodskaya Laboratoriya	Factory Laboratory
Zhur. Mikrobiol., Epidemiol. i Immunobiol.	Zhurnal Mikrobiologii, Epidemiologii i Immunobiologii	Journal of Microbiology, Epidemiology, and Immunobiology
Zhur. Nevropatol. i Psikhiat.	Zhurnal Nevropatologii i Psikhiatrii imeni S. S. Korsakov	S. S. Korsakov Journal of Neuropathology and Psychiatry
Zhur. Obshchei Biol.	Zhurnal Obshchei Biologiya	Journal of General Biology
Zhur. Vysshei Nerv. Deyatel.	Zhurnal Vysshei Nervnoi Deyatelnosti imeni I. P. Pavlova	I. P. Pavlov Journal of Higher Nervous Activity
Zool. Zhur.	Zoologicheskii Zhurnal	Journal of Zoology